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UNIVERSITY OF ALBERTA

— SYNTHESIS OF A POTENTIAL TRANSITION-STATE ANALOG FOR
DISACCHARIDE HYDROLYSIS

by —

(C) Mimi Bach

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA

SPRING, 1991



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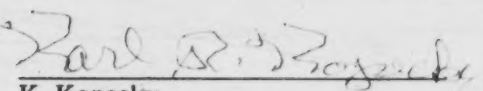
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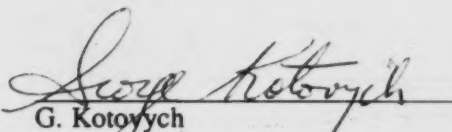
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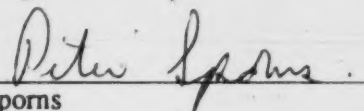
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The undersigned certify they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled, "Synthesis of a Potential Transition-State Analog for Disaccharide Hydrolysis", submitted by Mimi Bach, in partial fulfillment of the requirements for the degree of Master of Science in Organic Chemistry.


Ole Hindsgaul (Supervisor)


K. Kopecky


G. Kotovych


P. Sporns

Date: November 29, 1990

*To my husband, Quang, and my
children, Tammy and Phil*

ABSTRACT

Antibodies raised against transition state analogs of chemical reactions have been shown to sometimes catalyze the corresponding chemical reactions in solution. The acid-catalyzed hydrolysis of glycopyranosides is thought to proceed through flattened "half-chair" intermediates suggesting that flattened sugars might be transition-state analogs suitable for eliciting antibodies with "glycosidase" activity. To begin evaluating this hypothesis a flattened disaccharide glucal, namely 1,5-anhydro-3-O-[3-O-(4-carboxy-3-oxabutyl)-6-deoxy- β -D-glucopyranosyl]-2,6-dideoxy-D-arabino-hex-1-enitol, 32, has been chemically synthesized in a form suitable for attachment to protein carriers to produce an immunogen. The corresponding α and β disaccharides methyl 6-deoxy-3-O-[6-deoxy-3-O-(4-methoxycarbonyl-3-oxabutyl)- β -D-glucopyranosyl]- α,β -D-glucopyranoside, 31 and 30, the potential ground state ligands for the antibody, have also been prepared.

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A number of people have helped the author in various ways, and she wishes to thank them all, mentioning especially, Dr. U. Spohr for encouragement, Dr. R.M. Ippolito for assistance in the use of Chemdraw, Mrs. C. Elbrink for typing the manuscript, Dr. T.T. Nakashima and his associates for recording high field ^1H and ^{13}C nmr spectra, the Department of Chemistry of the University of Alberta, and the Natural Sciences and Engineering Research Council of Canada for financial support.

There is no sufficient way to thank my husband for his understanding and support.

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CHAPTER 1

Introduction

Enzymes are the major agents effecting controlled chemical changes in biological systems. Enzymes are generally characterized by their remarkable efficiency of catalysis and their extraordinary substrate specificity. Enzymes catalyze reactions by lowering the energy of the transition state, the activation energy, relative to that of the reactants or products.¹⁻³ To lower this activation energy, enzymes are thought to strongly bind to the transition state, a high energy configuration through which the reactant must pass to form products.

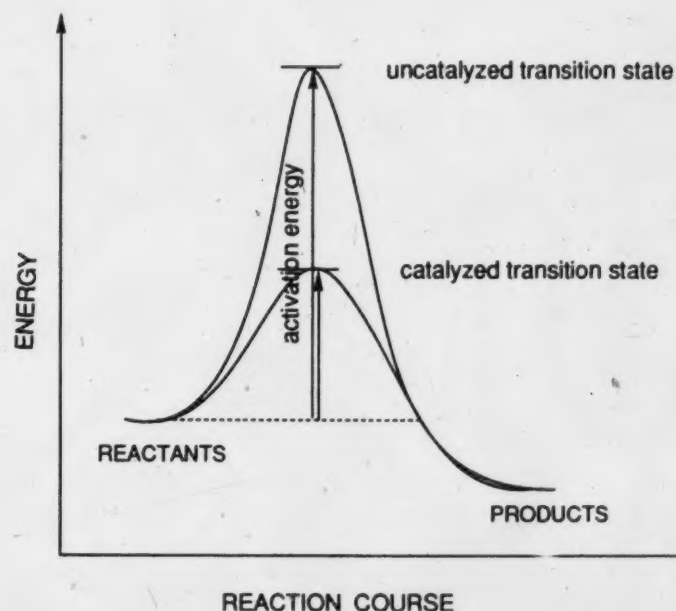


Fig. 1. Energy profile charts the energetic demands of a hypothetical chemical reaction, from reactants to products. The catalyst increases the rate of the chemical reaction by reducing the activation energy of the reaction.

Numerous examples in the literature confirm the usefulness of enzymes in biochemical and chemical reactions. To date hundreds of enzymes have been found and isolated from biological materials. However, enzymes are not available to catalyze all desired reactions and enzyme purification can be very difficult. Indeed, some enzyme purifications yield only microgram quantities of protein. The isolated proteins are furthermore often labile with respect to loss of catalytic activity.

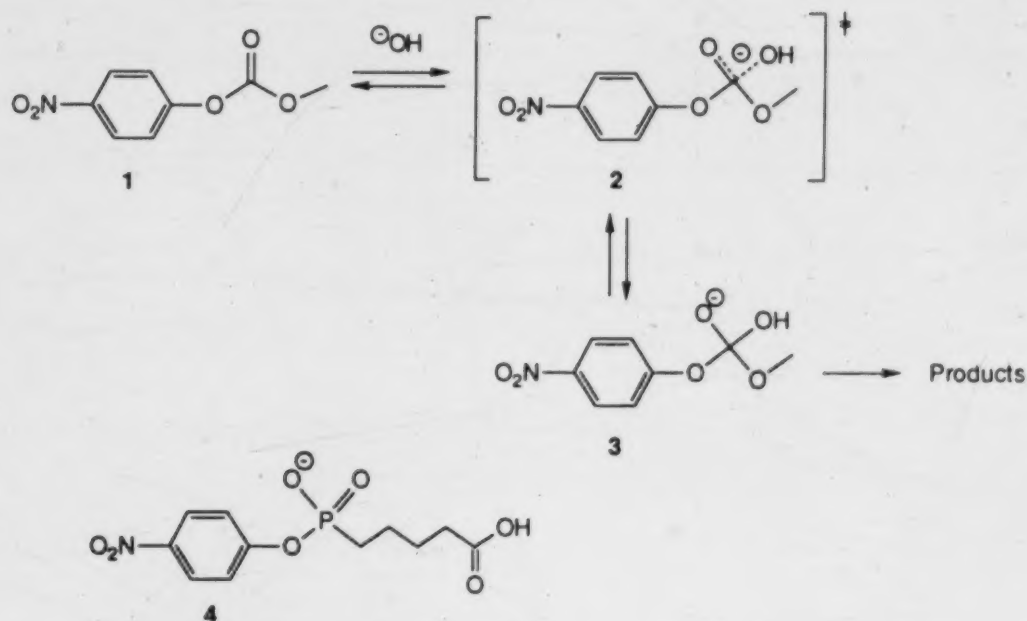
The similarity between antibodies and enzymes was first noted by Pauling.⁴ Antibodies are also proteins, but produced by the immune system in response to a challenge from potential invaders such as bacteria or viruses. The antibodies thus produced bind tightly to molecules on invading organisms, thereby initiating their destruction through a complex immune cascade.

Jencks⁵ first proposed that if one could develop an antibody to the transition state of a chemical reaction, that antibody might have a catalytic effect on the reaction. Obviously, transition states cannot be synthesized and isolated, but the synthesis of analogs of the transition state has been achieved successfully.

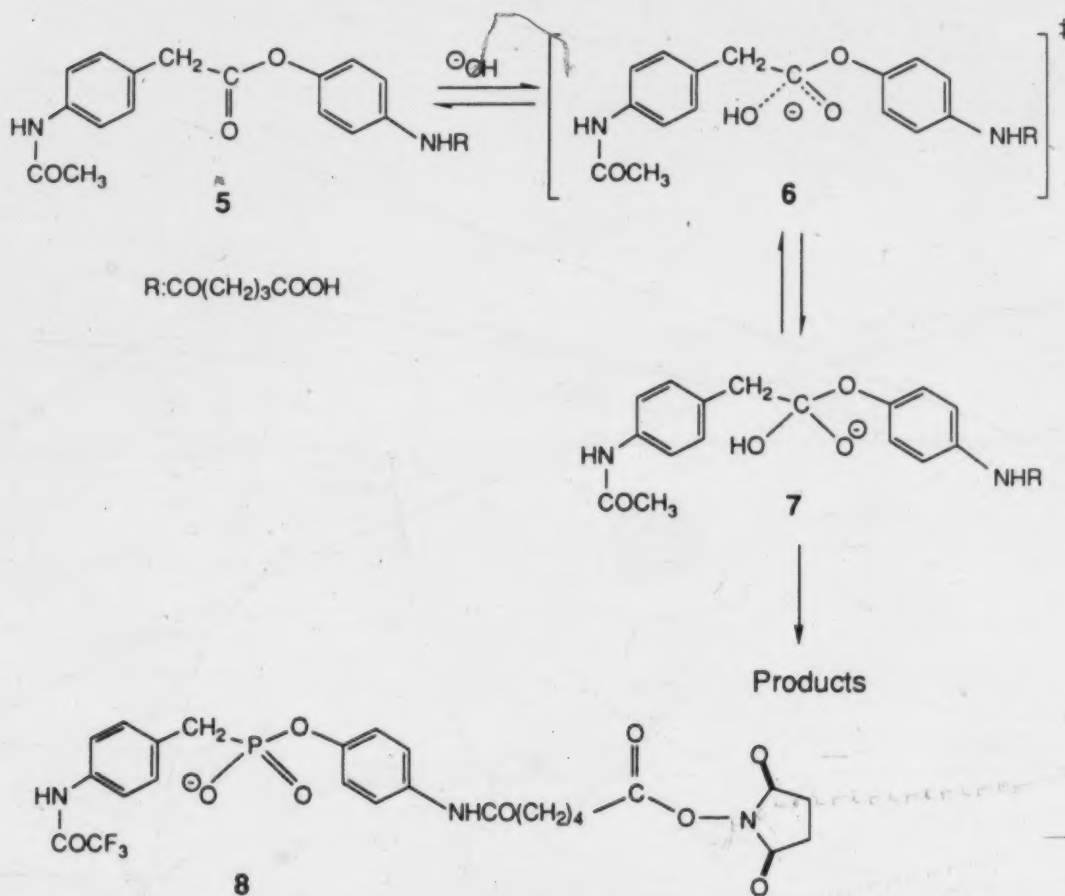
Schultz also suggested that "if an antibody binds a transition-state analog, it can then catalyze the reaction that goes through that transition-state".⁶ The antibody does that because, by binding the reactants in a configuration closely resembling the transition-state, it pushes the reactants toward the transition-state geometry. Current efforts in this regard focus on the development of strategies for introducing catalytic activity into antibody combining sites. Designed catalytic antibodies could have considerable value as therapeutic agents, catalysts for industrial chemical reaction, etc.

In the last five years, triumphs in the area of catalytic antibodies have been shared mostly by two groups of researchers working independently.⁷⁻¹² They have

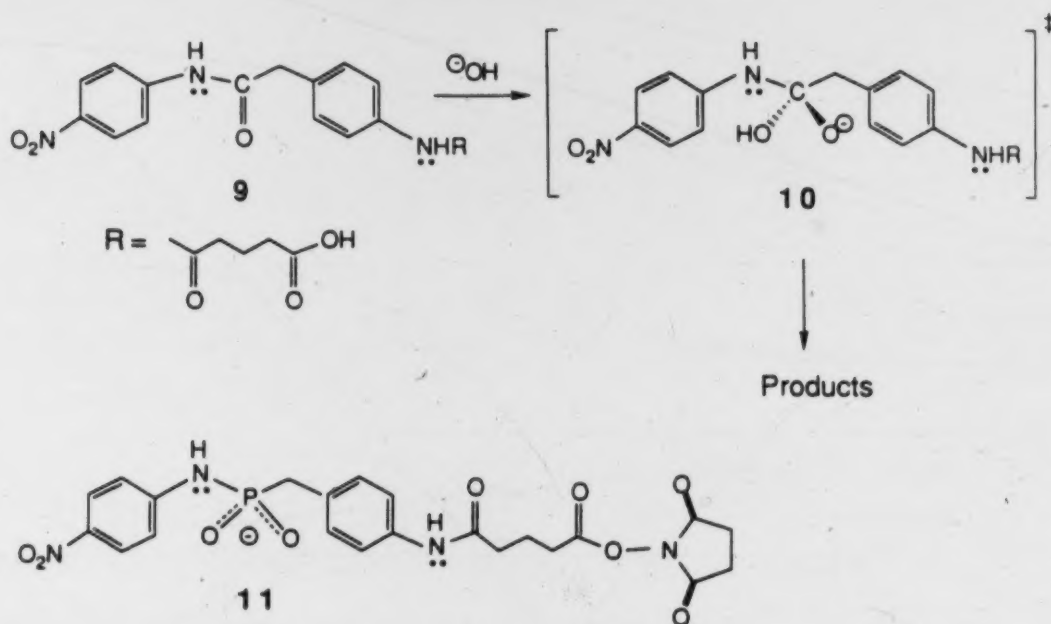
unequivocally shown that monoclonal antibodies raised against transition-state analogs can indeed catalyze reactions in the same manner as enzymes. The first successes in this area were the development of antibodies which showed acylase activity. The group of Schultz reasoned that the mechanism of alkaline hydrolysis of the *p*-nitrophenyl carbonate (1) proceeded through the transition state 2 to the tetrahedral intermediate 3; this was followed by collapse of 3 to form the products. They prepared the phosphonate-based transition state analog 4 for this reaction and produced monoclonal antibodies against this structure. One of these antibodies did indeed catalyze the hydrolysis of 1 with a rate enhancement of 810 over that of the uncatalyzed reaction.^{11,13,14}



Lerner and coworkers^{10,15-17} used a very similar approach but to prepare an antibody with esterase activity. The mechanism for the hydrolysis of ester **5** was expected to proceed through transition state **6** and to a tetrahedral intermediate (**7**) which would decompose to products. They prepared the transition state analog **8** which was used to immunize mice for the production of monoclonal antibodies (mAb).¹⁸ One of these antibodies, mAb 50D8, catalyzed the hydrolysis of **5** with a rate acceleration greater than 10^6 -fold over the uncatalyzed hydrolysis rate

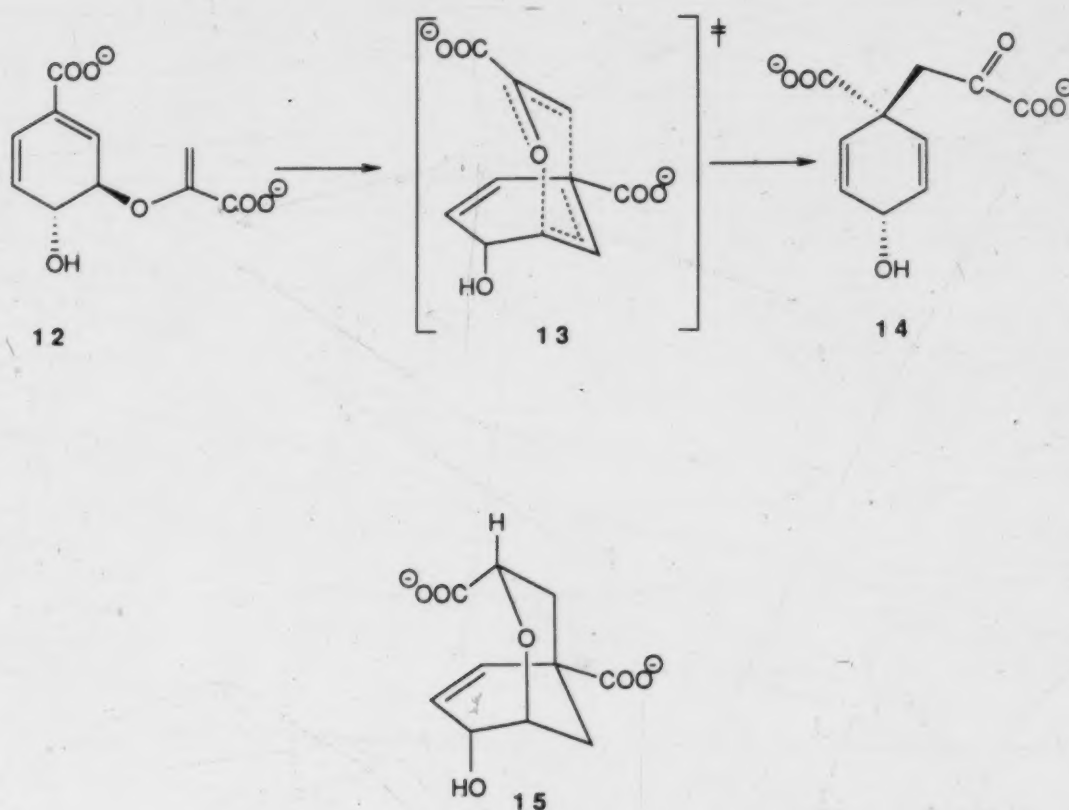


Using a similar approach, an antibody with amidase activity was produced.¹⁹ The hydrolysis of amide **9** is expected to proceed through transition state **10** for which arylphosphoramidate **11** was prepared as an analog. The antibody raised against **11** catalyzed the hydrolysis of carboxamide **9** with a large rate enhancement (250,000) relative to the uncatalyzed reaction.



As a final example of the scope of catalytic antibodies, Hilvert *et al.*^{20,21} developed an antibody capable of effecting a Claisen rearrangement.²²⁻²⁴ The antibody they produced showed chorismate mutase activity, catalyzing the conversion of chorismate **12** to prephenate **14**, a concerted reaction which is believed to proceed through transition state **13**. Antibodies elicited to the corresponding transition state analog **15** indeed catalyzed the rearrangement of **12** to **14** with the kinetic constants $k_{\text{cat}} = 1.2 \times 10^{-3} \text{ s}^{-1}$ and $K_{\text{M}} = 5.1 \times 10^{-5}$. The

transition state analog **15** was, as expected, a competitive inhibitor of the "enzyme" with a K_i value of $\approx 0.6 \times 10^{-6}$ M.



The above examples suggest that catalytic antibodies could, in principle, be produced that catalyze any desired chemical reaction, provided that a suitable transition state analog could be prepared. This thesis is concerned with the design and synthesis of such a transition state analog for glycoside hydrolysis,²⁵ a reaction fundamental to oligosaccharide and polysaccharide metabolism. The mechanism of acid catalyzed hydrolysis of glycopyranosides has been intensively studied^{26,27} and the generally accepted mechanism is shown in Fig. 2.

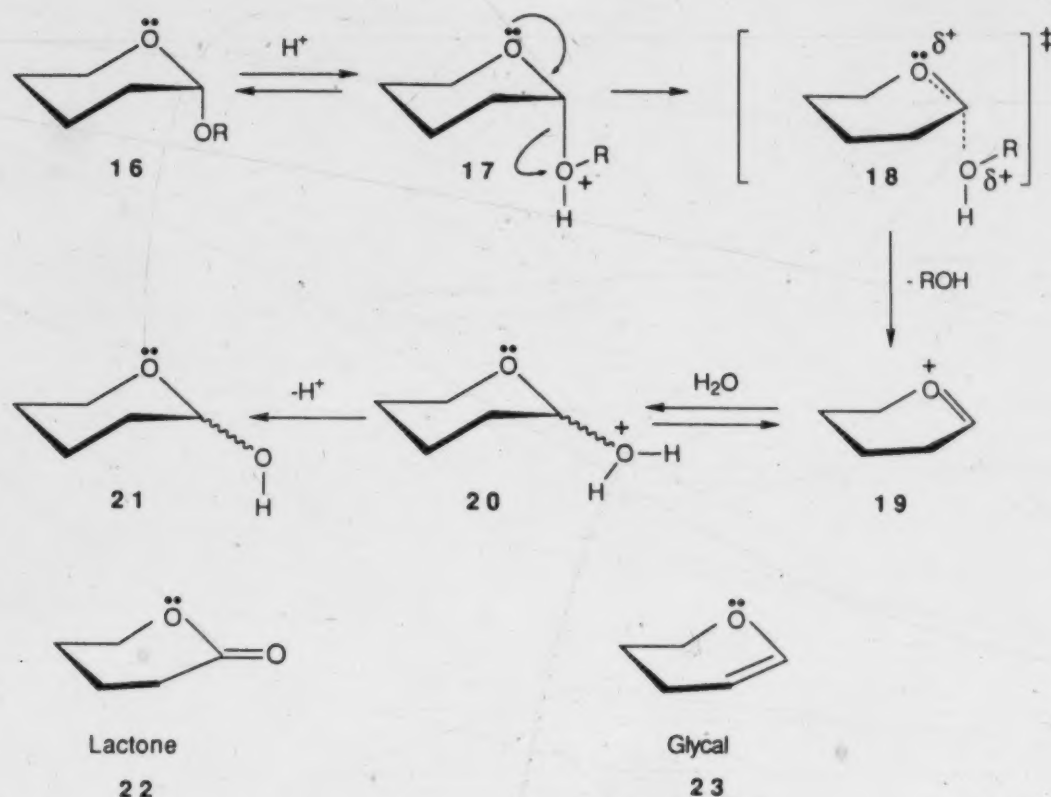


Fig.2. Mechanism of glycoside hydrolysis reaction:
Lactone **22** and glycal **23** are transition state analogs known in literature

In this solution mechanism, glycoside **16** undergoes rapid and reversible protonation of the aglyconic oxygen to produce **17** which decomposes, through participation of a lone-pair of electrons on the ring oxygen, to the oxocarbenium ion **19**. The transition state **18**, according to the Hammond postulate, is expected to resemble the high-energy intermediate **19**. Attack of water on **19** then gives **20** which can lose a proton to yield the reducing sugar **21**. A key feature of **19** (and

18) is that both the ring oxygen and anomeric carbon must approach sp^2 -hybridization in order for lone-pair stabilization to occur. This means that the pyranose ring in the transition state will distort from a chair form into a twisted or half-chair form to maximize the planarity required for resonance stabilization, as shown in Fig. 2. This argument is supported by the observation that sugar lactones **22** or glycals **23** frequently act as competitive inhibitors of glycosidases,^{28,29} presumably by mimicking the transition state. For example, β -D-galactal³⁰⁻³² and 1,5-gluconolactone^{27,28} are very powerful reversible inhibitors of β -galactosidase and glycogen phosphorylase, respectively. Furthermore, the high resolution crystal structure of the hexosamidase lysozyme has also been solved,^{27,33-36} both with and without a lactone inhibitor present, and the structure of the complex fully supports the notion that the flattened chair derivatives **22** and **23** do act as transition state analogs.

The work reported in this thesis represents the start of a program to develop antibodies with glycosidase activity. The objective here was not to immediately produce such antibodies but rather to examine whether antibodies could be produced against a flattened sugar structure and still cross-react with an "unflattened" sugar present in solution in the chair conformation. Both lactones **22** and glycals **23** were likely candidates in this regard since, as noted above, they appeared to function as transition state analogs against the naturally occurring enzymes. Many reports^{27,37,38} on the instability, with respect to hydrolysis and interconversions of sugar lactones of the type **22**, led us to the assumption that such structures were not likely to be useful as immunogens. The decision was therefore made to produce a glycal-based derivative for the attempted production of "sugar-flattening" antibodies. The glycoside model chosen in this work (**24**) is shown in Fig. 3.

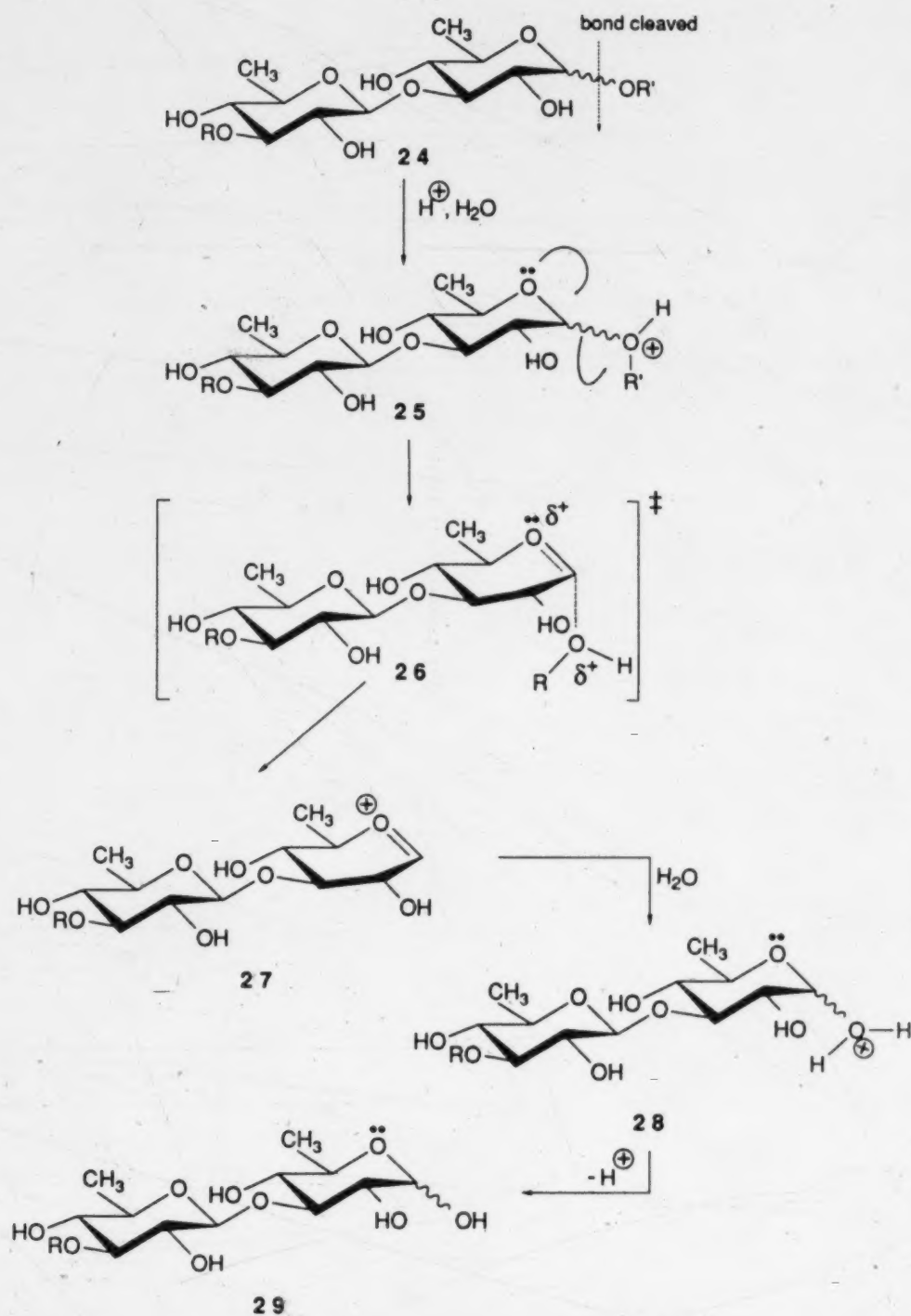
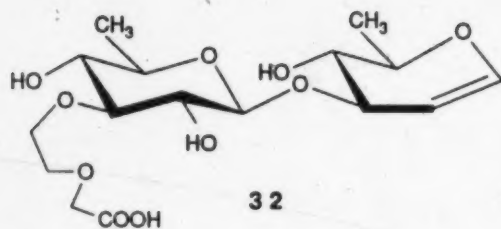
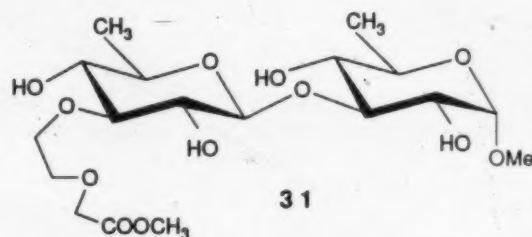
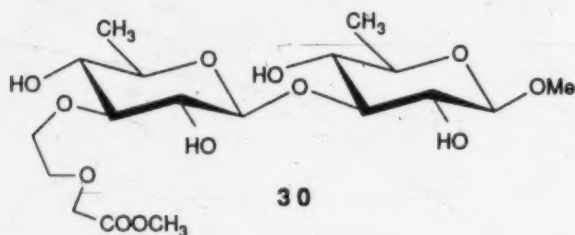


Fig. 3 Suggested Mechanism for the Acid Catalyzed Hydrolysis Reaction of Disaccharide 24.

The mechanism of cleavage of the aglycone (OR') in disaccharide **24** should, by analogy with other chemical and enzymatic hydrolyses, proceed *via* protonation of the glycosidic oxygen to give **25**. Decomposition of **25** through transition state **26** is then expected to lead to the flattened intermediate oxocarbenium ion **27**. Capture of **27** by water would give **28** which would lose a proton to yield **29**.

The α and β disaccharide methyl glycosides **30** and **31** were selected as the potential ground state ligands for the antibody and, correspondingly, the disaccharide glycal **32** was envisioned as an analog of the flattened oxocarbenium ion **27**, or transition state **26**.



The choice of compounds 30-32 was made for the following reasons: The immune system is incapable of producing antibodies against molecules as small as disaccharides unless these are attached to larger carriers, such as proteins. The 3'-carboxyl linking arm was therefore incorporated into 32 to allow its attachment to a carrier protein for subsequent immunization. The major decision was, however, the selection of 6-deoxy-D-glucose as the monosaccharide units. 6-Deoxy-D-glucose is not found in mammalian systems, but it does occur in bacteria. Our hope was that a good immune response would therefore be directed at "foreign" 32 and, furthermore, be directed not solely at the unnatural flattened glycal portion of the molecule, but also at the back side of the molecule; namely, against the side with the foreign methyl groups. The fact that the $\beta\text{Glc}(1\rightarrow3)\text{Glc}$ linkage is also not found in mammalian systems should further serve to render compound 32 immunogenic.

One additional feature for selecting 32 as the immunogen structure was based on the observations of Lemieux³⁹ that carbohydrate-binding proteins, including antibodies, frequently have hydrophobic pockets in which hydrophobic oligosaccharide surfaces become bound, surrounded by hydrophilic hydroxyl groups at the periphery of the combining site. Our expectation was that an antibody might be produced which agreed with Lemieux's ideas and therefore bind the side of the methyl groups at the bottom of the antigen-binding cleft. The CPK model of the HSEA-calculated^{40,41} minimum-energy conformer of 32 ($\phi = 55^\circ$, $\varphi = 5^\circ$, see Ref. 40) is shown below in Fig. 5, viewed both from the "front" (A) and "back" (B). When viewed from the back, the molecule appears to present a perfect hydrophobic surface which might be attractive for binding by the antibody. In this way, it was likely that the antibodies produced by immunization with 32 would also recognize the potential ground state ligands 30 and 31, by recognizing structural features

which are common to the transition-state analog **32** and ground state ligands **30** and **31**, a prerequisite for catalytic activity.

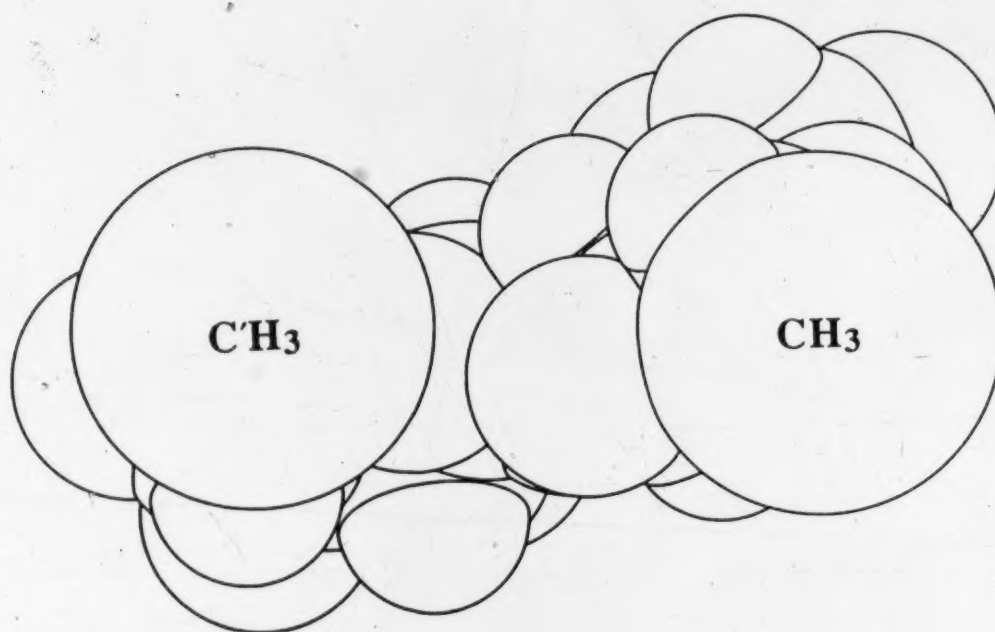


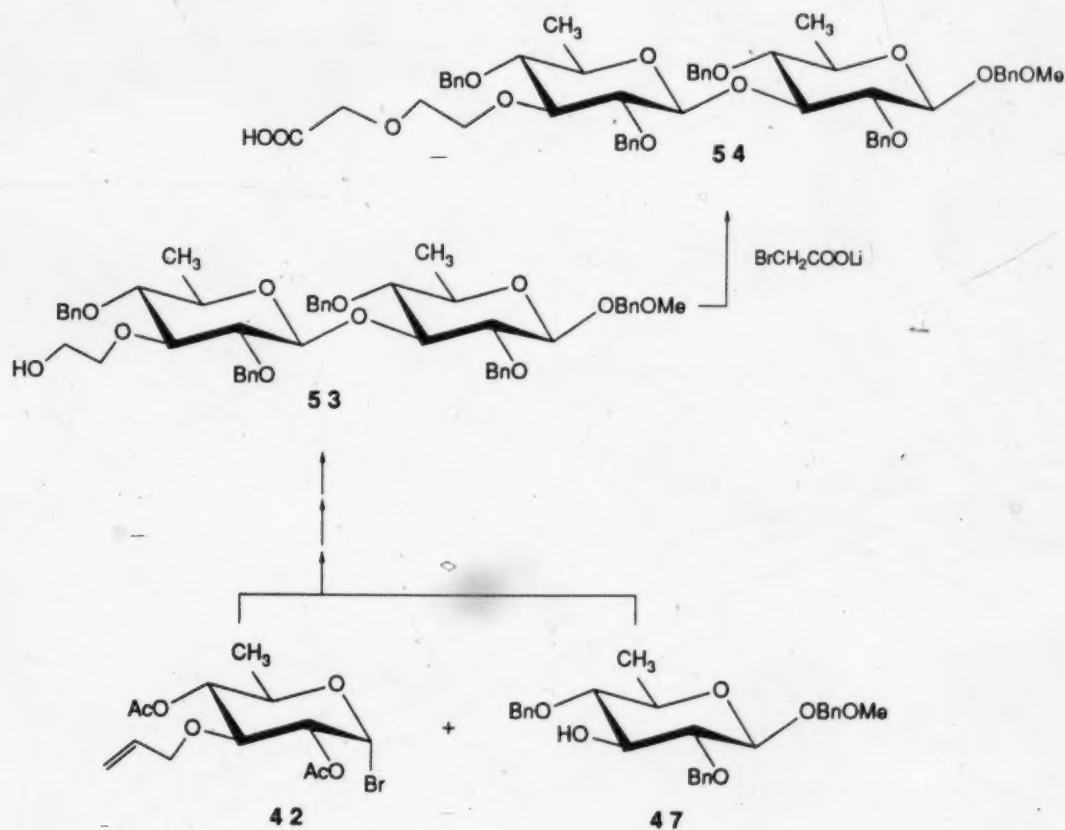
Fig. 5. CPK model of compound **32** viewed from the hydrophobic back side showing the two methyl groups.

Chapter 2

Results and Discussion

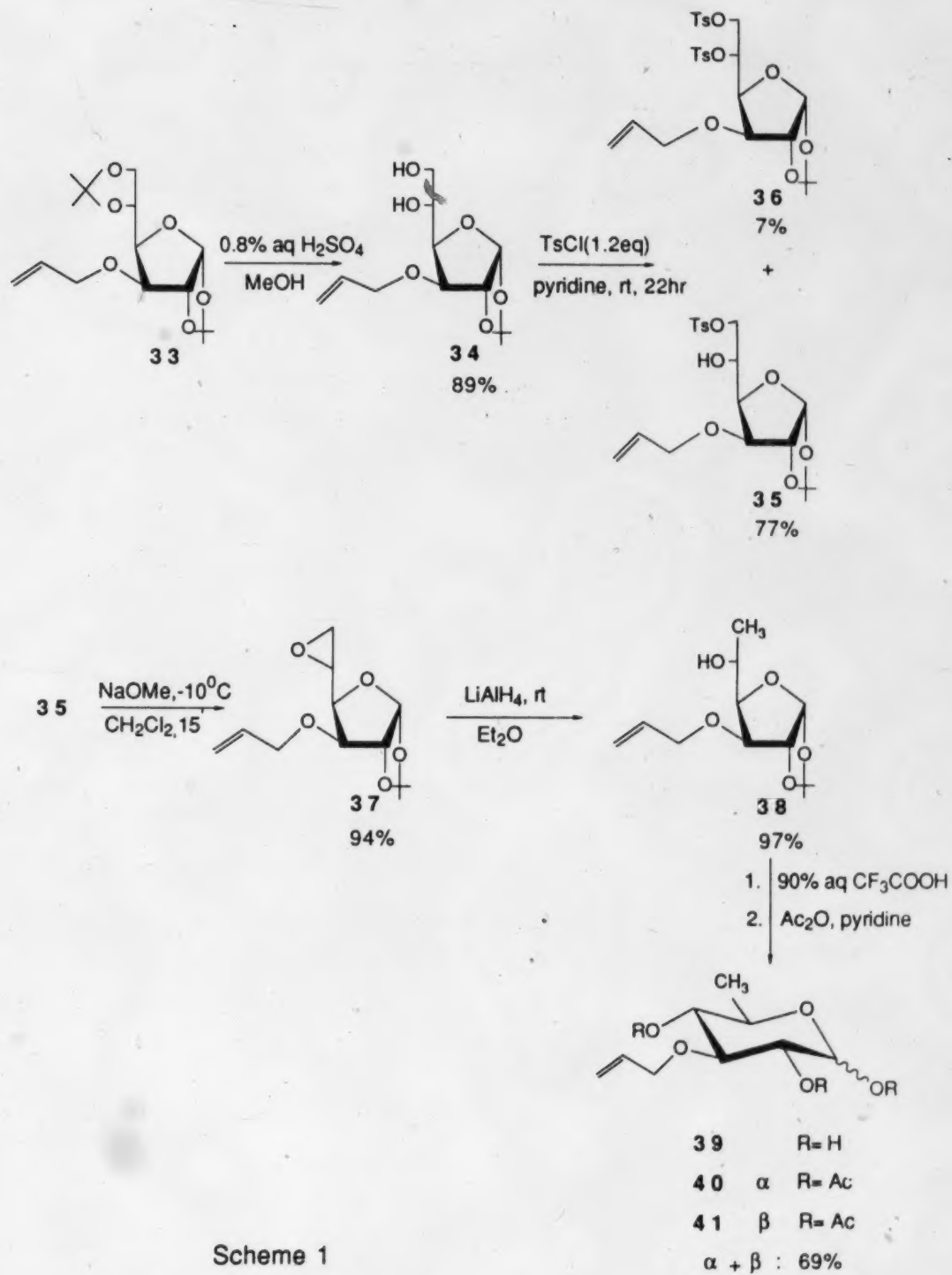
The objective of this thesis was to synthesize three disaccharides, namely **30** and **31** (potential substrates for flattening), and **32** (a transition-state analog for the hydrolysis of either **30** or **31**) (Fig. 4).

The key intermediate for the preparation of these target compounds was disaccharide **54**, which was prepared by coupling disaccharide **53** with bromoacetic acid, lithium salt.⁴² Disaccharide **53** was then retrosynthesized into two monosaccharide synthons **47** and **42**.



A synthetic route to the monosaccharide synthon **42** was first developed (Schemes 1 and 2). Compound **33** was selectively hydrolyzed to its 5,6-dihydroxy derivative **34**³⁸ in 89% yield on treatment with 0.8% aqueous sulfuric acid. Complete removal of the 5,6-*O*-isopropylidene ring of **33** was evident from the absence of the corresponding signals in the ¹H-nmr spectrum of **34**. The signals for H-6, H-6' appeared at δ 3.847 and 3.735, respectively. The signal for H-5 was observed at δ 4.232-4.023, though unresolved due to the overlapping with allylic protons, H-3, and H-4. Selective tosylation of the hydroxy group at C-6 with 1.24 molar equivalents of *p*-toluenesulfonyl chloride gave a mixture of mono- and ditosylates **35** and **36** (**35/36** $\approx$ 10/1). The reaction was therefore terminated before all **34** had reacted, thus allowing the isolation of pure **35** in 77% yield. The ¹H-nmr spectrum of **35** showed the expected downfield signals for H-6 at δ 4.285 and H-6' at δ 4.097. In agreement with the structural assignment, the ¹³C-nmr spectrum revealed a signal at δ 72.40 for C-6.

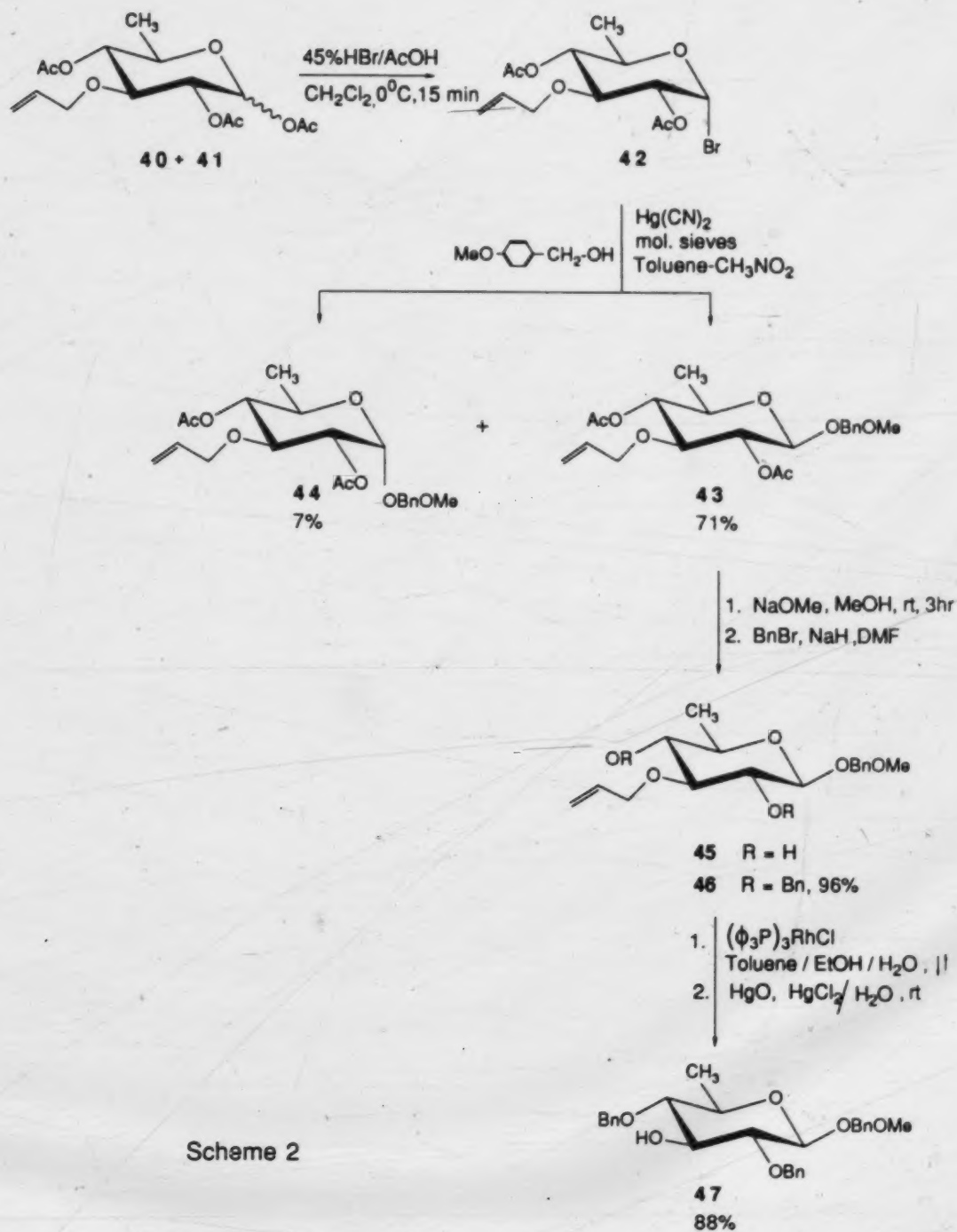
The reaction of **35** with sodium methoxide, following the procedure of Meyer & Reichstein⁴⁴ resulted in the formation of **37** in 94% yield. As expected, the ¹H-nmr spectrum showed no aromatic proton signals, but upfield shifted signals at δ 2.921 and 2.779 for H-6 and H-6', respectively. Treatment of epoxide **37** with lithium aluminium hydride in dry diethyl ether at room temperature effected the reductive opening of the epoxide ring to provide the 6-deoxy derivative **38** in 97% yield. The structure of **38** was confirmed by its ¹H- and ¹³C-nmr spectra. A doublet proton signal for the three protons of C-6 and a doublet signal for the hydroxylic proton of C-5 were observed at δ 1.325 (d, $J_{5,6}$ = 6.5 Hz) and 2.510 (d, $J_{OH,5}$ = 6.7 Hz), respectively.



Scheme 1

The opening of the 1,2-isopropylidene ring of **38** with aqueous trifluoroacetic acid (90%) provided 3-*O*-allyl-6-deoxy- α - and - β -*D*-glucopyranose **39**. Compound **39** was not isolated, but acetylated to give a mixture of α and β anomers of 1,2,4-tri-*O*-acetyl-3-*O*-allyl-6-deoxy- α,β -*D*-glucopyranose **40** and **41** as solids in 69% yield. Each anomer was obtained in pure form after chromatographic separation. In their ^1H -nmr spectra, proton signals for H-1 α and H-1 β were observed at δ 6.24 (d, $J_{1,2} = 3.8$ Hz) and 5.63 (d, $J_{1,2} = 8.5$ Hz), respectively. Their optical rotations are $[\alpha]_D + 64.0^\circ$ (c 0.57, CHCl_3 , α anomer) and $[\alpha]_D + 13.2^\circ$ (c 0.54, CHCl_3 , β anomer). Reaction of triacetates **40** and **41** in dichloromethane with 45% hydrogen bromide in acetic acid solution at 0°C led to the formation of the glycosyl bromide **42** in essentially quantitative yield. Bromosugar **42** was a key intermediate in the preparation of synthon **47** and disaccharide **52**.

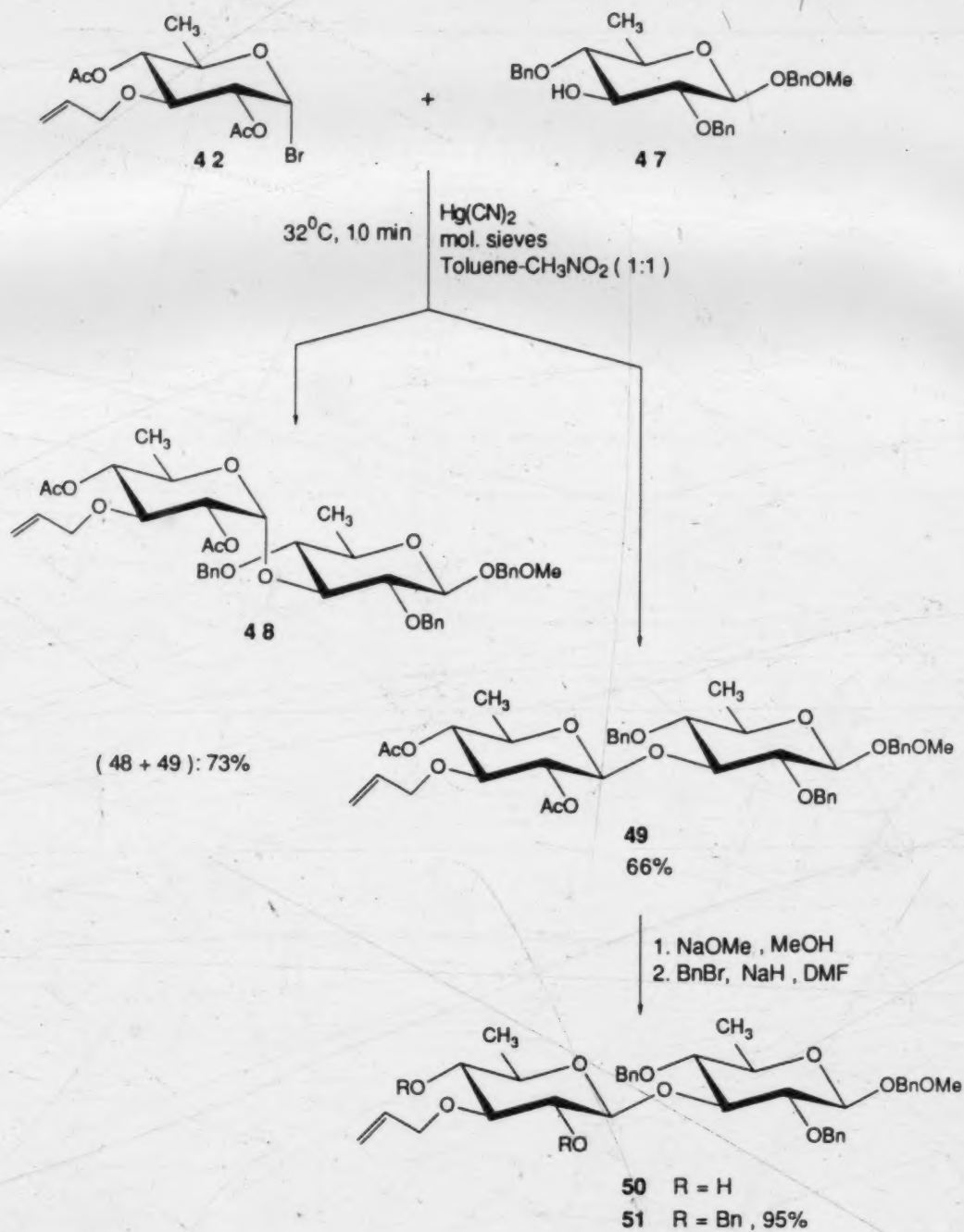
The monosaccharide synthon **47** was prepared as follows (Scheme 2). Reaction of 4-methoxybenzyl alcohol with 2,3-di-*O*-acetyl-3-*O*-allyl-6-deoxy- α -*D*-glucopyranosyl bromide **42** in acetonitrile using mercury(II) cyanide and mercury(II) bromide as promoter at room temperature, as described by Tahir and Hindsgaul,⁴⁵ provided the desired product although the yield of 32% proved unacceptably low. In an attempt to accomplish the preparation of **43** in higher yield, the conditions described by Srivastava and Hindsgaul⁴⁶ were followed. Reaction of 4-methoxybenzyl alcohol with bromide **42** using silver trifluoromethane sulfonate/tetramethylurea as promoter in the presence of 4 Å molecular sieves in CH_2Cl_2 at -30°C for the first 0.5 h and then 4 h at room temperature provided the monosaccharide **43** in 44% yield. This increase in the yield of **43** was not yet satisfactory. Finally, Helferich conditions⁴⁷ using only mercury(II) cyanide as promoter in the presence of 4 Å molecular sieves in toluene-nitromethane solution



Scheme 2

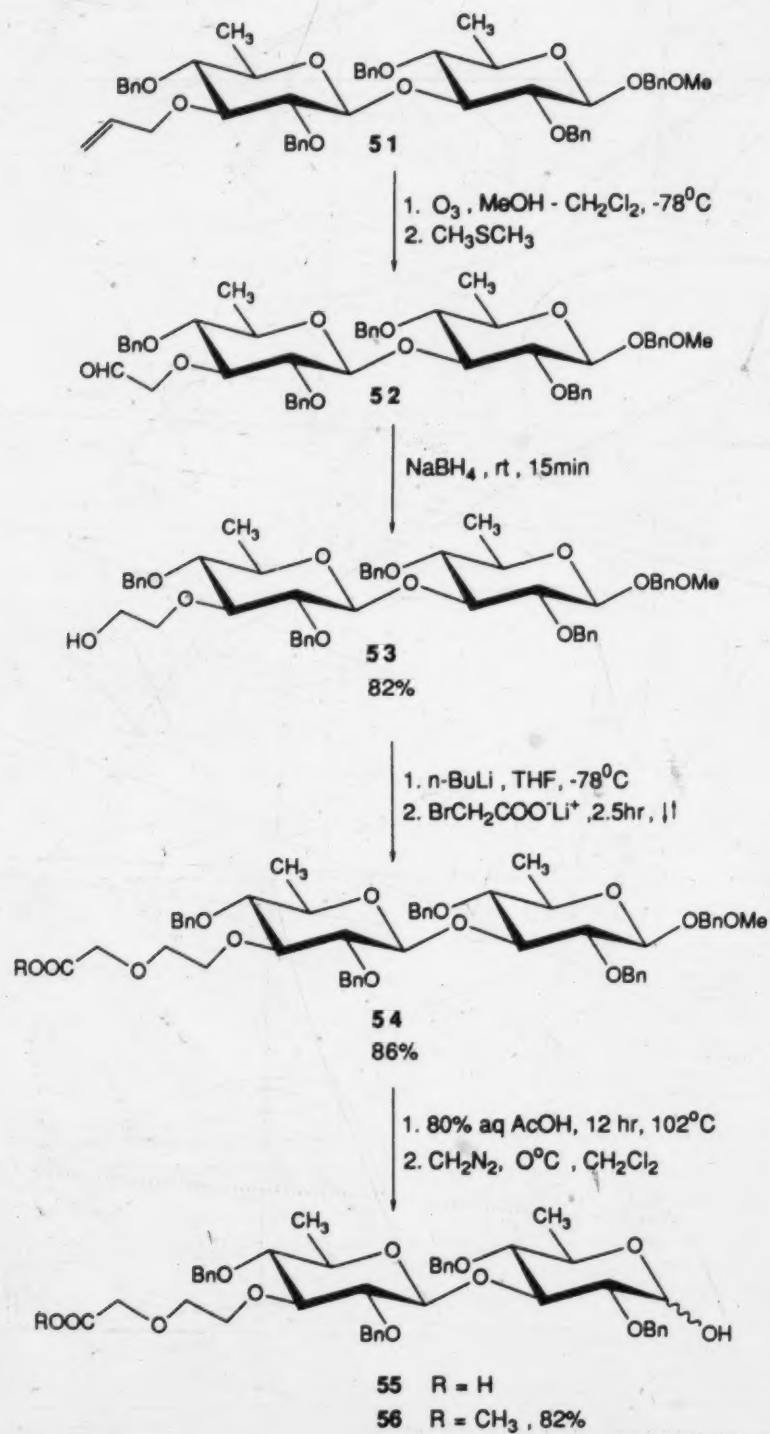
(1:1) at room temperature were employed to afford the desired product, compound **43**, in 71% yield. The formation of the β linkage in **43** was confirmed by the ^1H -nmr spectrum which showed a doublet proton signal for H-1 β at δ 4.388 ($J_{1,2} = 8.0$ Hz). The introduction of a new protecting group, 4-methoxybenzyl, at the anomeric center was proven by the appearance of new signals at δ 7.211, 6.870 (aromatic protons), 4.800, 4.528 (2d, benzylic protons) and 3.808 (s, OCH_3). The undesired α -anomer **44**, was isolated in 7% yield. The structure of this minor product could also be confirmed by its ^1H -nmr spectral data, which indicated a doublet for H-1 α at δ 5.203 ($J_{1,2} = 4.0$ Hz), about 0.820 ppm downfield relative to H-1 in the corresponding β anomer **43**. Removal of the acetyl groups of compound **43** was accomplished by treatment with sodium methoxide in methanol to provide the diol **45**. Benzylation of **45** with benzyl bromide-sodium hydride afforded compound **46** in 96% yield. The structure of **46** was determined on the basis of ^1H -nmr and ^{13}C -nmr spectral data. The nmr spectra of **46** confirmed the disappearance of the acetyl groups and their replacement with benzyl groups. Finally, deallylation of **46** was accomplished in a two step reaction with tris(triphenylphosphine)rhodium(I)chloride as promoter according to the procedure developed by Corey and Suggs,⁴⁸ which effected the isomerization of the double bond to provide the prop-1-enyl-ether which, on treatment with aqueous acetone-mercuric chloride in the presence of mercuric oxide,⁴⁹ underwent hydrolysis to afford the desired monosaccharide alcohol **47** in 88% yield. The removal of the allyl group was confirmed by ^1H -nmr and ^{13}C -nmr spectral data. Specially, the appearance of a D_2O -exchangeable one proton doublet at δ 2.43 ($J_{3,\text{OH}} = 2.0$ Hz) further supported the assignment of the structure **47** to the product.

Coupling of freshly prepared bromosugar **42** with alcohol **47** was attempted using $\text{AgOSO}_2\text{CF}_3$ -TMU, powdered 4 Å molecular sieves in dichloromethane. Although **42** was used in an excess amount, the glycosylation did not go to completion as indicated by the presence of unreacted **47** ($\approx 41\%$) on the tlc plate. The expected product **49** was obtained in only 52% yield. No α anomer was detected. However, by using $\text{Hg}(\text{CN})_2$ and powdered 4 Å molecular sieves in toluene-nitromethane (1:1) at 30-35°C, a mixture of α and β anomers could be isolated in 73% yield ($\alpha/\beta = 1/10$). The reaction progressed much faster under these conditions, but decomposition occurred due to the acid lability of the products at this somewhat elevated temperature. It was therefore critical to terminate the reaction quickly. Unreacted alcohol **47** was recovered in 26% yield. The desired β anomer **49** was isolated in 66% yield. The configuration of the newly formed glycosidic linkage in **49**, expected to be β due to neighboring group participation of the 2-acetoxy group in the glycosylation reaction, was evident from its ^1H -nmr spectrum which showed a doublet proton signal for H-1' at δ 4.969 ($J_{1',2'} = 8.0$ Hz). Treatment of **49** with sodium methoxide in methanol allowed the removal of the acetyl groups to give compound **50**. This compound was not characterized, but its structure was confirmed on the basis of the ^1H -nmr spectrum which showed the disappearance of two singlets for the acetyl methyl protons. Benzylolation of **50** with benzyl bromide-sodium hydride in DMF provided compound **51** in 95% yield. The process to synthesize compound **51** is outlined in Scheme 3. The presence of benzyl groups at O-2' and O-4' was confirmed by the appearance, in the ^1H -nmr spectrum, of additional signals integrating for 10 protons in the aromatic region, and additional benzylic protons at δ 4.887-4.486. The ^{13}C -nmr spectral data were also in agreement with the assigned structure.



Scheme 3

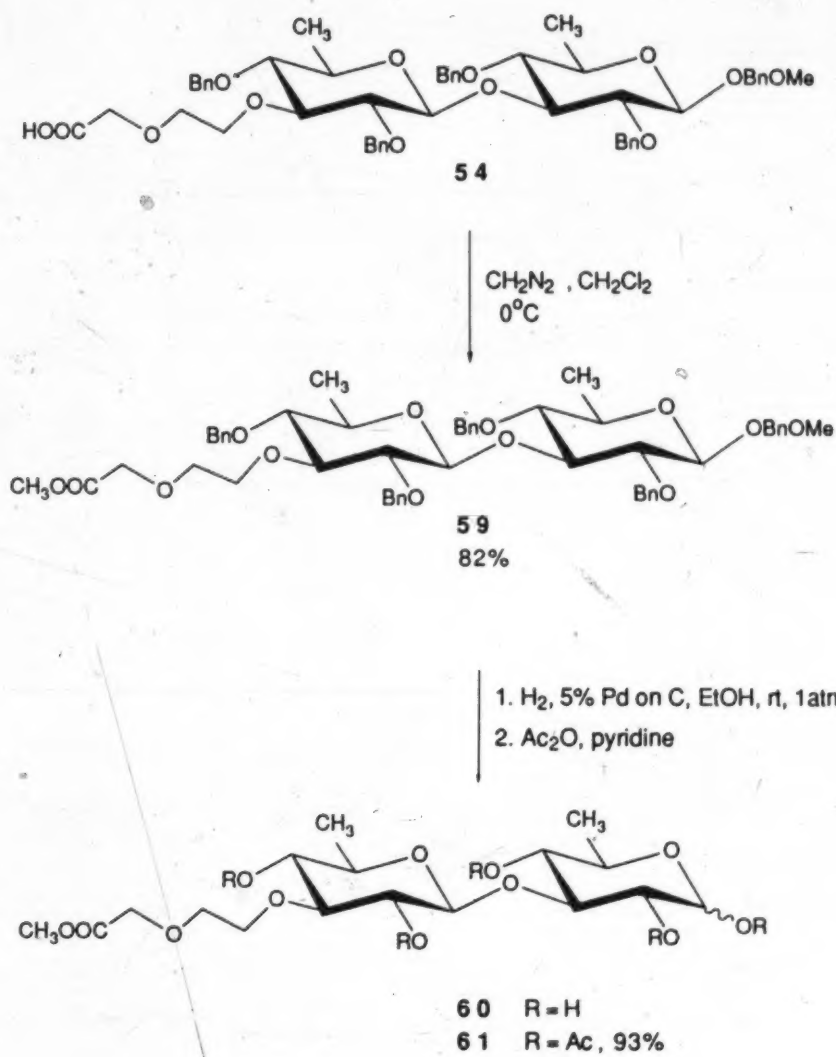
Conversion of **51** to **53** was achieved in two steps without characterization of the intermediate **52** involved (Scheme 4). Ozonolysis of **51**⁵⁰ with ozone gas in dry methanol-dichloromethane (1:1) at -78°C for 5 minutes followed by treatment with dimethyl sulfide gave the aldehyde **52** whose structure was determined by its ^1H -nmr spectrum. The aldehyde proton appeared as a triplet signal at δ 9.620 ($J = 0.9$ Hz). Aldehyde **52** was reduced immediately with NaBH_4 to the corresponding alcohol **53** in 82% yield. The structure of **53** could be deduced easily from its ^1H -nmr spectrum, which displayed the absence of all allyl proton signals, the presence of four protons of $\text{OCH}_2\text{CH}_2\text{O}$ at δ 3.912, 3.671, and the presence of a hydroxylic proton at δ 2.940 (t, $J_{\text{OH},\text{CH}_2} = 6.5$ Hz). The assignment of structure **53** was also supported by the ^{13}C -nmr spectral data. No allyl carbon signals were observed, but the presence of new signals at δ 70.97, 62.91, respectively, was assigned to $\text{OCH}_2\text{CH}_2\text{OH}$. Bromoacetic acid lithium salt, used in the preparation of **54**, was prepared by neutralization of bromoacetic acid with aqueous lithium hydroxide (0.01 N). To remove any remaining trace of acid, the crude salt was exhaustively washed with dried diethyl ether. Reaction of **53** with bromoacetic acid lithium salt in THF using $n\text{-BuLi}$ as a base to generate the alkoxide at -78°C as described by Tenbrink⁴² provided the expected product **54** in 86% yield after neutralization and chromatography. Although the acid proton could not be detected in the ^1H -nmr spectrum, the structure was supported by ^{13}C -nmr spectral data. New signals at δ 172.17 ($-\text{COOH}$) and 71.95 (OCH_2COOH) were observed. Furthermore, the microanalytical data were in accord with the molecular formula for **54**. Use of sodium hydride in toluene as the base in the above reaction gave only a low yield of **54**.



Scheme 4

Attempted selective removal of the 4-methoxybenzyl group from compound **54** using either dichlorodicyanoquinone (DDQ),⁵¹ or N-bromosuccinimide (NBS) as described by Classon et al.,⁵² or by cerium(IV) ammonium nitrate (CAN) reagent as described by Johansson and Samuelsson,⁵³ failed to provide **55**. However, treatment of **54** with 80% aqueous acetic acid at 102°C did give reducing **55**. The progress of the reaction was difficult to monitor because compound **54** and **55** had the same chromatographic mobility on tlc. When *p*-anisidine phthalate solution (0.10 M) was used in the place of H₂SO₄-EtOH solution (5:95) to spray the plate, compound **55** could clearly be distinguished by the dark colour of its charring. Meanwhile compound **54** did not char. Compound **55** was not fully characterized, but the ¹H-nmr spectra confirmed the absence of the 4-methoxybenzyl group by the disappearance of the signal for three protons of the methoxy group at δ 3.743 and the decrease in intensity of the signals for aromatic and benzylic protons. Esterification of **55** using diazomethane in dichloromethane provided the desired product **56** in 82% yield. Reaction of reducing disaccharides **56** with methyl iodide in toluene using silver oxide as promoter at room temperature gave **57** and **58** in 73% and 10% yield, respectively. The structure of **57** as the β anomer was assigned on the basis of its ¹H-nmr spectrum which showed H-1 as a doublet at δ 4.227 ($J_{1,2} = 8.0$ Hz). The corresponding α -anomer **58** had H-1 at δ 4.371 with $J_{1,2} = 4.0$ Hz.

Hydrogenolytic cleavage of the benzyl protecting groups of **57** using 5% palladium-on-charcoal as a catalyst in 98% ethanol furnished the target disaccharide **30** which was purified by chromatography on silica gel. Pure **30** was obtained as a thick syrup in 95% yield. The ¹H-nmr spectrum of **30** exhibited the expected signals for H-1 at δ 4.670 ($J_{1,2} = 7.5$ Hz), H-1' at δ 4.367 ($J_{1',2'} = 8.0$ Hz).



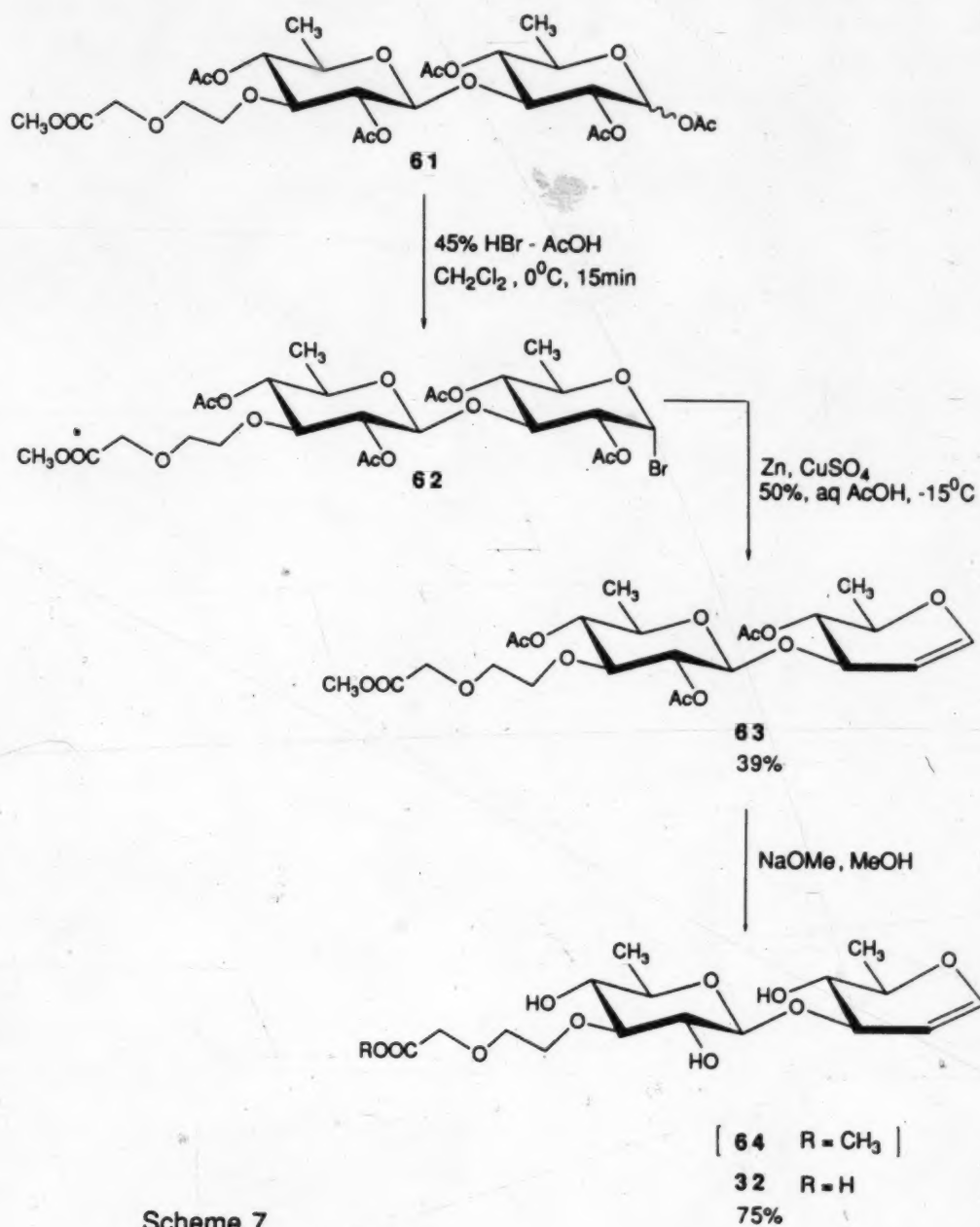
Scheme 6

Other signals were compatible with this structural assignment. Further proof for the assigned structure **30** was provided by its ^{13}C -nmr spectral data and the microanalytical data. Disaccharide **31** was similarly obtained from **58**. The structure of **31** was assigned on the basis of its ^1H -nmr spectral data which showed two one proton doublet signals at δ 4.741 ($J_{1,2} = 3.0$ Hz) for H-1, 4.632 ($J_{1',2'} = 7.5$ Hz) for H-1'.

As seen in Scheme 6, treatment of acid **54** with diazomethane in dichloromethane at 0°C afforded the corresponding ester **59** in 82% yield. The formation of the ester was confirmed by the appearance of a singlet at δ 3.743 for three methoxy protons in the ^1H -nmr spectrum of the product and signals at δ 170.79 (COOCH_3) and 51.73 (OCH_3) in its ^{13}C -nmr spectra.

Debenzylation of **59** afforded a mixture of α and β anomers of compound **60**. On treatment of the mixture with acetic anhydride-pyridine solution (1:2), **61** was produced in 93% yield. Replacement of all benzyl and 4-methoxybenzyl groups with acetyl groups was confirmed by ^1H -nmr spectroscopy. The integration of the signals between δ 2.166 and 2.061 requires the presence of five acetyl groups (15 H). The absence of aromatic and benzylic protons was also observed. Furthermore, the microanalytical data were in accord with the molecular formula for compound **61**.

Treatment of **61** in dichloromethane with a 45% solution of hydrogen bromide in glacial acetic acid at 0°C led to the quantitative formation of **62** the assigned structure of which was supported by its ^1H -nmr spectrum. The expected downfield shifted signal for H-1 at δ 6.570 (d, $J_{1,2} = 4.0$ Hz) confirmed the substitution of the acetyl group by a bromide at C-1. Elimination of OAc and Br substituents from glycosyl bromide **62**, performed as described⁵⁴ by using zinc dust



Scheme 7

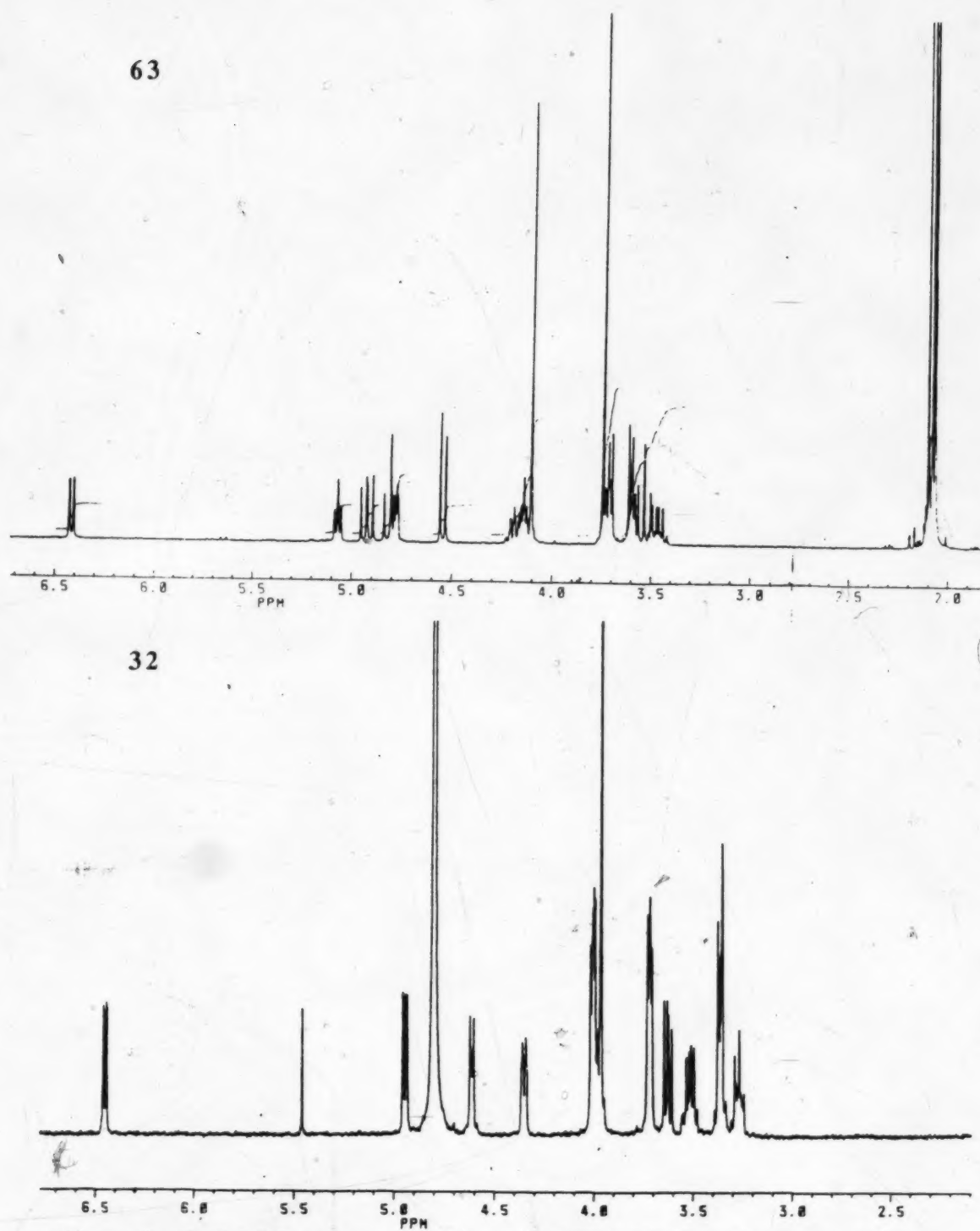


Figure 6. Partial ^1H -nmr spectra of compound 63 in CDCl_3 and compound 32 in D_2O .

and cupric sulfate pentahydrate in 50% aqueous acetic acid at -15°C , provided glycal **63** in 39% yield (Scheme 7). The formation of the double bond at C-1 and C-2 was determined by ^1H -nmr and ^{13}C -nmr spectral data. The absence of one acetyl group was observed and large downfield shifts exhibited by the anomeric proton and H-5. Long range couplings were found between H-1 and H-3 ($J_{1,3} = 1.3$ Hz), H-2 and H-4 ($J_{2,4} = 0.7$ Hz), H-3 and H-5 ($J_{3,5} = 1.3$ Hz). Furthermore, the molecular weight of **63** was supported by mass spectral data. The ^1H -nmr spectrum is reproduced in Figure 6. Removal of the acetyl protecting groups from **63** was accomplished by treatment with sodium-methoxide in methanol. Filtration of the crude product through a column of Bio-Gel P-2 afforded the target disaccharide **32** as a white lyophilized powder in total yield of 75%. Complete removal of the acetyl groups was evident from the absence of the corresponding signals in its ^1H -nmr spectrum. The signals for H-1, H-1' appeared at δ 6.451 ($J_{1,2} = 6.3$ Hz, $J_{1,3} = 1.3$ Hz), 4.611 ($J_{1',2'} = 8.0$ Hz), respectively. The ^1H -nmr spectrum is presented in Figure 6.

The coupling constants for the ring protons of compounds **30** and **31** provide evidence that their pyranoid rings exist in the $^4\text{C}_1$ conformation (Table 2). As expected, the values of the diaxial coupling constants are in the normal range (8-12 Hz) while axial-equatorial coupling constants are much smaller. A comparison of coupling constants for compound **32** with those for compounds **30** or **31** reveals that the glycal moiety of **32** is not in the $^4\text{C}_1$ conformation. As shown in Table 2 and in Figures 7 and 8, the value of $J_{3,4\text{ax}}$ is smaller than the accepted range (8-12 Hz) for diaxial coupling. Indeed, the coupling constants for **32**, i.e. $J_{1,2}$, $J_{1,3}$, $J_{2,3}$, $J_{3,4}$ (6.3, 1.3, 2.6 and 6.7 Hz, respectively) are in accord with the coupling constants published for D-glucal (6.1, 1.6, 2.2 and 6.8 Hz, respectively).⁵⁵

Furthermore, the chemical shifts of the ring protons (Table 1) and the coupling constants for the glucal portion of triacetate **63** are very similar to those reported for tri-*O*-acetyl-D-glucal in the literature.^{56,57} Indeed, the conformation of triacetyl-D-glucal has been established as being ⁴H₅ by X-ray crystallography,⁵⁸ and by ¹H-nmr spectroscopy.⁵⁶ Therefore, the glycal moiety of **32** is seen to be in essentially the same flattened conformation as D-glucal. The structure of compounds **30**, **63**, 3,4,6-tri-*O*-acetyl-D-glucal, D-glucal and **32** are shown in Figure 9.

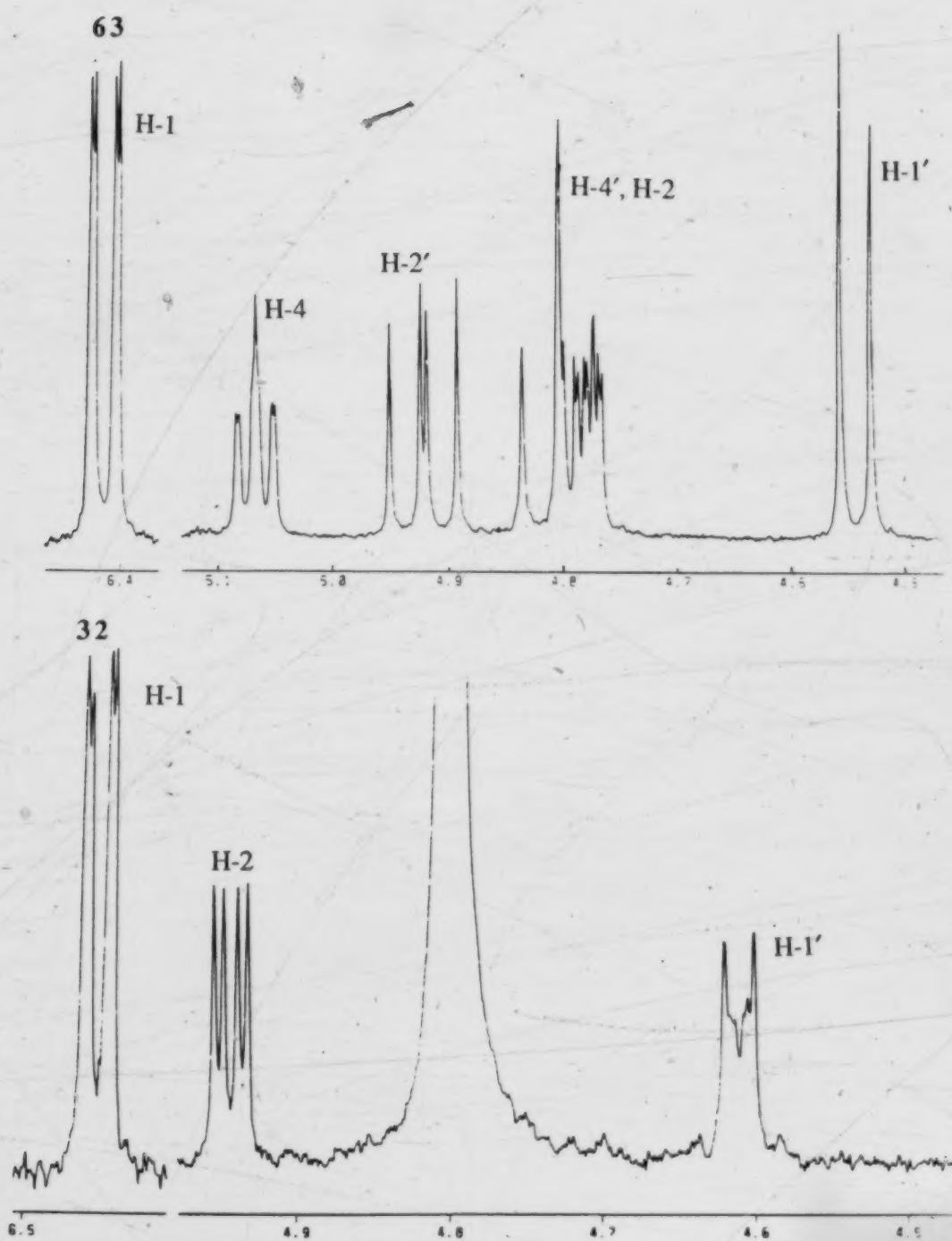


Figure 7. ^1H -nmr spectra (only anomeric region shown) of compound 32 in D_2O and 63 in CDCl_3 .

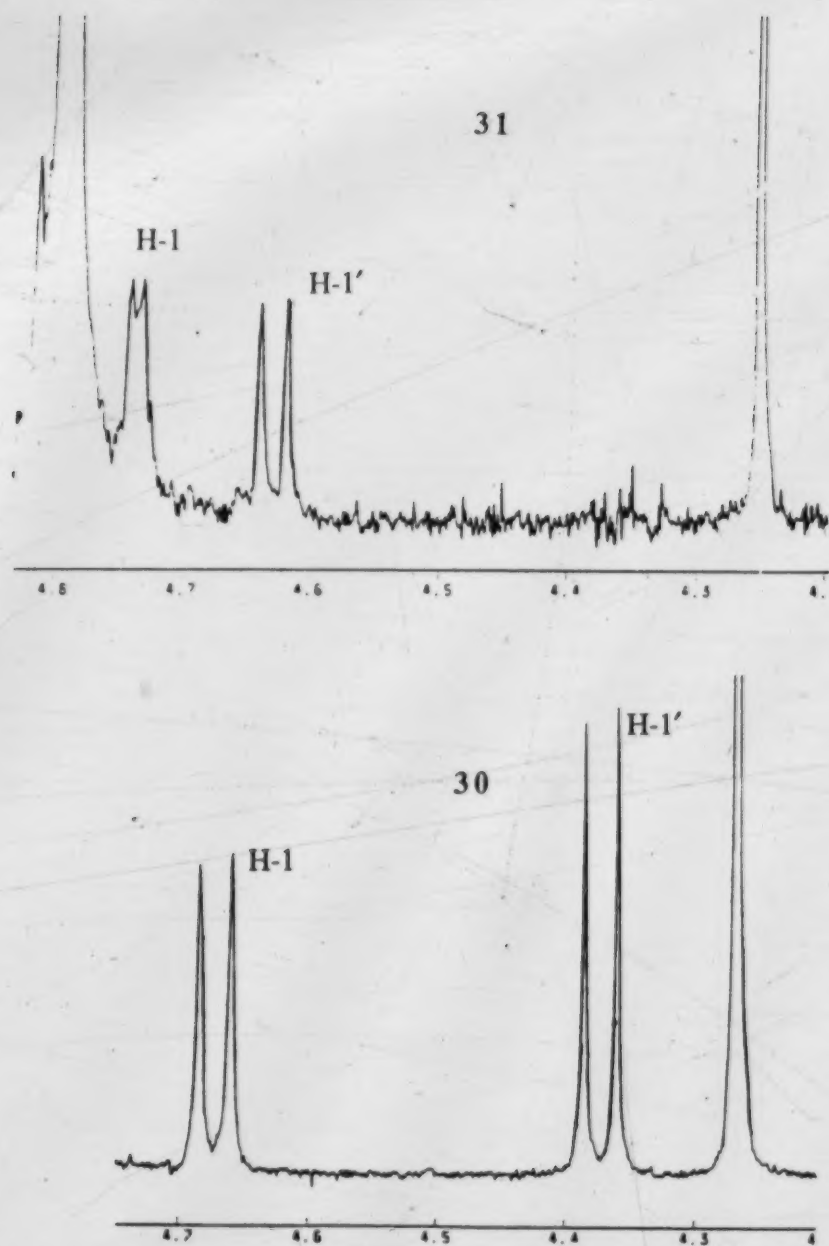


Figure 8. ^1H -nmr spectra (only anomeric region shown) of compound 31 and 30 in D_2O .

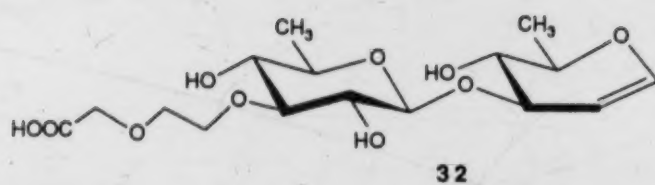
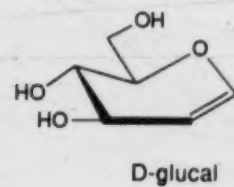
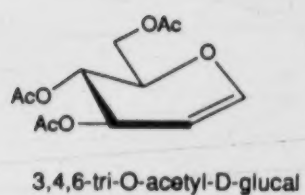
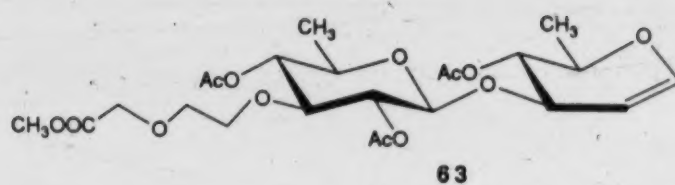
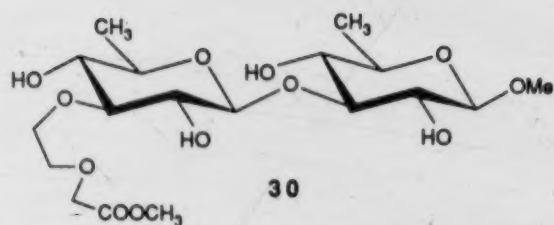


Figure 9. Structure of compounds 30, 63, 3,4,6-tri-O-acetyl-D-glucal, D-glucal and 32.

Table 1.

Comparison of selected ^1H -nmr chemical shifts (δ , ppm) of **63** and tri-*O*-acetyl-D-glucal^a in CDCl_3 , and of **32** and D-glucal^b in D_2O .

Proton	30	63	Tri- <i>O</i> -acetyl-D-Glucal	32	D-glucal
H-1	4.67	6.42	6.53	6.45	6.86
H-2	n.d.	4.78	4.81	4.94	5.26
H-3	3.64	4.14	5.31	4.35	4.65
H-4	3.23	5.07	5.20	3.63	4.14
H-5	n.d.	4.19	4.19	n.d.	4.35
*H-6a	1.30	1.29	4.09	1.36	4.31
*H-6b			4.29		4.31
H-1'	4.37	4.54		4.61	
H-2'	3.44	4.92		n.d.	
H-3'	3.36	3.53		n.d.	
H-4'	3.26	4.80		n.d.	
H-5'	n.d.	3.46		n.d.	
H-6'	1.28	1.21		1.302	

^aSpectral data from Hall and Johnson.⁵⁶

^bSpectral data from Korytnyk et al.⁵⁵

***63** and **32** are 6-deoxy compounds, H-6a, H-6b, H-6c are equivalent.

n.d., not determined.

Table 2.

Comparison of selected ^1H -nmr coupling constants (J , Hz) of **63** and tri-*O*-acetyl-D-glucal^a in CDCl_3 , and of **32** and D-glucal^b in D_2O .

J	30	63	Tri- <i>O</i> -acetyl-D-Glucal	32	D-glucal
1,2	7.5	6.3	6.4	6.3	6.1
1,3		1.3	1.3	1.3	1.6
2,3	9.2	3.75	3.2	2.6	2.2
2,4		0.70			
3,4	9.5	4.0	6.4	6.7	6.8
3,5		1.3			
4,5	9.5	4.75	6.8	9.2	n.d.
5,6	6.0	6.7	6.3	6.2	n.d.
5,6b			2.4		n.d.
6,6b			14.0		n.d.
1',2'	8.0	8.0		8.0	
2',3'	9.0	9.2		n.d.	
3',4'	9.0	9.4		n.d.	
4',5'	n.d.	9.4		9.2	
5',6'	6.0	6.2		6.2	

^aSpectral data from Hall and Johnson.⁵⁶

^bSpectral data from Korytnyk et al.⁵⁵

n.d., not determined.

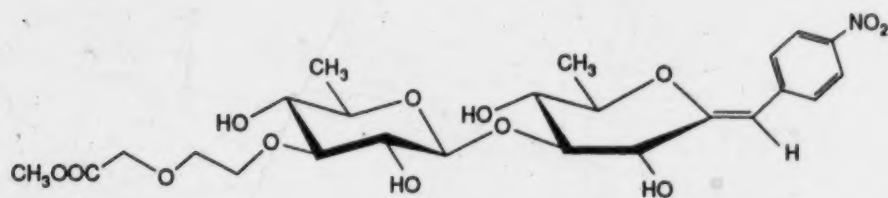
CHAPTER 3

Future Prospects

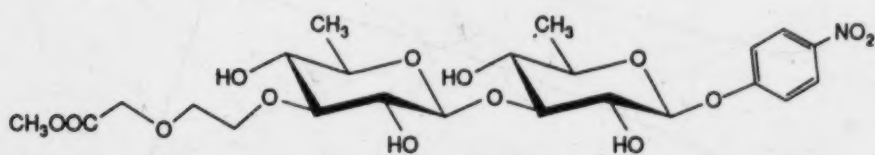
At the time of this writing, antibodies directed against **32** have not yet been produced. Compound **32** has, however, been coupled through its carboxyl group to the lysine amino groups of bovine serum albumin (BSA) using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and a BSA-antigen with an incorporation of 5 has been prepared. Attempts to increase this incorporation to a more desirable 15-20 ligands/BSA are in progress. Rabbit immunizations are about to begin and the plan is to follow the protocol of Lemieux et al. for the production and affinity isolation of anti-carbohydrate antibodies.⁵⁹

Since disaccharide **32** contains features which are expected to be immunogenic it is likely that high-affinity antibodies against **32** will be formed provided that this disaccharide, with a chemically labile enol-ether grouping, survives the antigen presentation process in the animal. The likelihood that anti-**32** antibodies will either bind to, flatten or even catalyze the hydrolysis of either **30** or **31** is a matter for speculation. In the event that the isolated anti-**32** antibodies do not possess any of these desired properties, which may well prove to be the case, the conclusion would be that there is insufficient structure in common between **32** and the two "ground-state" glycosides for a cross-reaction to occur. A proposed solution to the production of the desired catalytic antibodies would then be to use a glycal-type analog which would provide more structure in common with the ground state glycosides so that binding of this additional structure could provide sufficient energy to produce a measurable cross-reaction and therefore, potentially, flattening and hydrolysis. The exocyclic glycal **65** might be a reasonable candidate in this

regard since it would extend the hydrophobic surface of the back-side of the disaccharide to include the hydrophobic nitrophenyl group. Antibodies produced against **65** might then have sufficient affinity for the non-reducing terminal sugar residue and aromatic "aglycone" in **65** to bind with the glycoside **66** and consequently distort the reducing-end sugar towards the shape of the glycal, i.e. to move the conformation of **66** towards that of the presumed transition state for hydrolysis. Inclusion of the nitro group in **65** and **66** would furthermore facilitate the assay for glycosidase activity since the release of *p*-nitrophenol would result in the production of a yellow color.



65



66

CHAPTER 4

Experimental

General methods - All solvents and reagents used were reagent grade and, in cases where further purification was required, standard procedures⁶⁰ were followed. All solid reactants for glycosylation reactions were dried overnight over phosphorus pentoxide in a high vacuum prior to use. Solution transfers where anhydrous conditions were required were done under argon using standard syringe techniques.⁶¹ Molecular sieves were purchased from BDH Chemicals. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at ambient temperatures ($22 \pm 2^\circ$).

Thin-layer chromatography (tlc) was performed on precoated plates of silica gel (60-F254, Merck) with detection by quenching of fluorescence, or by charring, or both, after spraying with 5% sulfuric acid in ethanol. Unless otherwise stated, column chromatography was performed on Silica Gel 60 (Merck, 230-400 mesh) and distilled solvents were used. For gel filtration, Bio-Gel P-2 (200-400 mesh) (Bio-Rad Laboratories) was used.

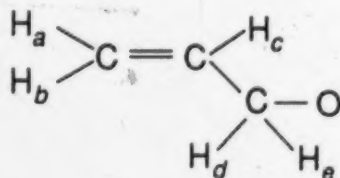
Proton nuclear magnetic resonance (^1H -nmr) spectra were recorded at either 400 MHz (Bruker WH-400) or 300 MHz (Bruker AM-300) with either tetramethylsilane ($\delta = 0$ in CDCl_3) or acetone ($\delta = 2.225$ in D_2O) as internal standard at ambient temperature. Carbon-13 nuclear magnetic resonance (^{13}C -nmr) spectra were recorded at 75.5 MHz (Bruker AM-300 or Bruker WM-360) with internal tetramethylsilane ($\delta = 0$ in CDCl_3) or external 1,4-dioxane ($\delta = 67.4$ in D_2O) as reference standards. The ^1H -nmr chemical shifts and coupling constants are

reported as though they were first order. Assignments of ^{13}C resonances are tentative. The microanalyses were carried out by the Analytical Services Laboratory of this Department.

The following solvent systems have been designated by letters:

A, ethyl acetate-hexane (1:1, v/v); B, ethyl acetate-hexane (1:2); C, ethyl acetate-hexane (2:5); D, ethyl acetate-hexane (1:3); E, ethyl acetate-hexane (1:4); F, ethyl acetate-hexane (1:5); G, ethyl acetate-hexane (1:7); H, ethyl acetate-hexane (2:1); I, ethyl acetate-hexane (5:1); J, diethyl ether-hexane (1:1); K, acetone-hexane (1:3); L, acetone-hexane (2:7); M, methanol-dichloromethane (2:3); N, methanol-dichloromethane (1:19); O, ethyl acetate-dichloromethane-hexane (1:1:5); P, ethyl acetate-dichloromethane-hexane (1:1:8); Q, ethyl acetate-dichloromethane-hexane (2:1:2); R, ethyl acetate-dichloromethane-hexane (6:3:6); S, ethyl acetate-dichloromethane-hexane (1:1:15); T, ethyl acetate-dichloromethane-hexane (1:1:12); U, ethyl acetate-dichloromethane-hexane (1:1:10); V, methanol-dichloromethane-acetic acid (10:190:1).

Protons of the allyl group present in the compounds described in this work were designated as *Ha*, *Hb*, *Hc*, *Hd* and *He* as defined below.



These protons showed the same multiplicity pattern in all the compounds examined and only the chemical shifts varied. The observed coupling constants were:

Ha, dddd, $J_{a,c} = 10.5$ Hz

$J_{a,b} = 1.5 \pm 0.5$ Hz

$J_{a,d} = J_{a,e} = 1.5 \pm 0.5$ Hz

Hb, dddd, $J_{b,c} = 17.0$ Hz

$J_{b,a} = 1.5 \pm 0.5$ Hz

$J_{b,d} = J_{b,e} = 1.5 \pm 0.5$ Hz

Hc, dddd, $J_{c,a} = 10.5$ Hz

$J_{c,b} = 17.0$ Hz

$J_{c,d} = J_{c,e} = 5.5$ Hz

Hd, dddd, $J_{d,e} = 13.0$ Hz

$J_{d,c} = 5.5$ Hz

$J_{d,a} = J_{d,b} = 1.5 \pm 0.5$ Hz

He, dddd, $J_{e,d} = 13.0$ Hz

$J_{e,c} = 5.5$ Hz

$J_{e,a} = J_{e,b} = 1.5 \pm 0.5$ Hz

3-O-Allyl-1,2;5,6-di-O-isopropylidene- α -D-glucofuranose (33). - Allyl bromide (12.5 mL; 144.5 mmol) was added to a stirring mixture of 1,2;5,6-di-*O*-isopropylidene- α -D-glucofuranose (15 g, 57.6 mmol), barium oxide (39.16 g; 255.0 mmol), barium hydroxide octahydrate (11.56 g; 37.0 mmol) and *N,N*-dimethylformamide (135 mL) at room temperature. After 18 h, insoluble materials were filtered off and washed with dichloromethane (750 mL). The combined filtrate

was washed with water (4 x 200 mL). Solvent evaporation followed by chromatographic purification (solvent G) of the residue gave compound **33** (15.93 g, 92%) as syrup; R_f : 0.33 (solvent G); its characterization has been reported in the literature.⁶²

3-O-Allyl-1,2-O-isopropylidene- α -D-glucofuranose (34). - Compound **33** (21.31 g, 71.0 mmol) was dissolved in methanol (98 mL) and 0.8% aqueous sulfuric acid by weight (98 mL) was added. After 15 h at room temperature, barium carbonate (4.0 g) and water (250 mL) were added and the reaction mixture was boiled for seven minutes and solids were removed by filtration. The filtrate was washed with dichloromethane (200 mL x 7) and taken to dryness. The residual syrup was purified by chromatography on silica gel using solvent D as eluent. Pure **34** (16.45 g; 89%) was obtained as a syrup; R_f : 0.19 (solvent B); $[\alpha]_D -50.3^\circ$ (c 0.49, chloroform); $^1\text{H-nmr}$ (CDCl_3): δ 5.907 (m, 1H, Hc allyl), 5.930 (d, 1H, $J_{1,2} = 4.0$ Hz, H-1) overlapping with Hd, 5.322 (1H, Hb allyl), 5.243 (1H, Ha allyl), 4.568 (d, 1H, $J_{2,1} = 3.7$ Hz, H-2), 4.232-4.023 (m, 5H, Hd and He allyl, overlapping with H-3, H-4, H-5), 3.847 (dd, 1H, $J_{6,6'} = 11.5$ Hz, $J_{6,5} = 3.5$ Hz, H-6), 3.735 (dd, 1H, $J_{6',6} = 11.5$ Hz, $J_{6',5} = 5.5$ Hz, H-6'). $^{13}\text{C-nmr}$ (CDCl_3): δ 133.69 ($\text{CH}_2=\text{CH}-\text{CH}_2\text{O}$), 118.19 ($\text{CH}_2=\text{CH}-\text{CH}_2\text{O}$), 111.85 ($\text{O}-\text{C}(\text{CH}_3)_2-\text{O}$), 105.14 (C-1), 82.29, 82.19, 80.01, 69.52 (all methine, C-2,3,4,5), 71.10 ($\text{CH}_2=\text{CH}-\text{CH}_2\text{O}$), 64.52 (C-6), 26.81, 26.88 ($\text{O}_2\text{C}(\text{CH}_3)_2$).

Anal. calc. for $\text{C}_{12}\text{H}_{20}\text{O}_6$: C, 55.36; H, 7.76. Found: C, 55.08; H, 7.41.

3-O-Allyl-1,2-O-isopropylidene-6-O-p-toluenesulfonyl- α -D-glucofuranose (35). - To a cold solution of compound **34** (33.12 g, 127.0 mmol) in dry pyridine

(100 mL), a solution of *p*-toluenesulfonyl (tosyl) chloride (30 g, 157.0 mmol) in dry dichloromethane (180 mL) was added dropwise at 0°C under argon atmosphere. After the addition, stirring was continued for 22 h at room temperature, by which time tlc indicated the presence of two less polar products, the major product: R_f : 0.36 (solvent A), compound **35**, the minor product R_f : 0.45 (solvent A), most likely compound **36** ($\approx$ 5%), and the presence of unreacted compound **34** ($\approx$ 10%), R_f : 0.06 (solvent A). The excess of tosyl chloride was decomposed by the addition of water (100 mL) and the mixture was left for 1 h at room temperature. Removal of solvent led to a brown residue. Pyridine was removed by coevaporation with toluene (200 mL x 4). The remainder was dissolved in dichloromethane (700 mL) and washed with water (100 mL x 4), 5% hydrochloric acid solution (100 mL) and water (100 mL). The organic solution was dried (Na_2SO_4) and concentrated to provide a yellow syrup which was purified by chromatography on silica gel using solvent A as eluent. Pure **35** (40.56 g; 77%) was obtained as a syrup, R_f : 0.47 (solvent B); $[\alpha]_D -14.8^\circ$ (c 0.55, chloroform). ^1H -nmr (CDCl_3): δ 7.804, 7.352 (4H, aromatic), 5.870 (1H, Hc allyl), 5.863 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1) overlapping with Hc allyl, 5.297 (1H, Hb allyl), 5.222 (1H, Ha allyl), 4.547 (d, 1H, $J_{2,1} = 3.5$ Hz, H-2), 4.285 (dd, 1H, $J_{6,6'} = 10.0$ Hz, $J_{6,5} = 2.5$ Hz, H-6), 4.180 (ddd, 1H, $J_{5,6} = 2.5$ Hz, $J_{5,6'} = 6.0$ Hz, $J_{5,4} = 10.0$ Hz, H-5) partially overlapped with Hd allyl, 4.167-4.017 (5H, Hd and He overlapping with H-4, H-3 and H-6 (δ 4.097, dd, $J_{6'6} = 10.0$ Hz, $J_{6,5} = 6.0$ Hz)), 2.462 (s, 3H, $\text{CH}_3\text{C}_6\text{H}_4$), 1.460, 1.310 (2s, each s, 3H, $\text{O}_2\text{C}(\text{CH}_3)_2$); ^{13}C -nmr (CDCl_3): δ 145.06 ($\text{C}_6\text{H}_4\text{SO}_2$), 133.66 ($\text{CH}_2=\text{CH}-\text{CH}_2\text{O}$), 132.62 ($\text{CH}_3-\text{C}_6\text{H}_4$), 129.99, 128.11 (C_6H_4), 118.18 ($\text{CH}_2=\text{CH}-\text{CH}_2\text{O}$), 111.99 ($\text{O}_2\text{C}(\text{CH}_3)_2$), 105.16 (C-1),

82.22, 81.89, 79.21, 67.43 (all methine C-2, 3,4,5), 72.40 (C-6), 71.34 (CH₂=CH-CH₂O); 26.83, 26.88 (all methyl of O₂C(CH₃)₂), 21.69 (C₆H₄CH₃).

Anal. calc. for C₁₉H₂₆O₈S: C, 55.06; H, 6.34. Found: C, 54.93; H, 6.08.

3-O-Allyl-5,6-anhydro-1,2-O-isopropylidene- α -D-glucofuranose (37). - A cold methanolic 2.89 N sodium methoxide solution (22 mL) was added to a solution of compound **35** (10.36 g, 25.0 mmol) in dry dichloromethane (32 mL) at -10°C to -15°C. The reaction mixture was stirred at the same temperature for 15 minutes. During that period the reaction mixture gradually solidified as a jelly. The mixture liquified again as the temperature was raised to room temperature within 30 minutes while stirring. Tlc then showed the complete disappearance of compound **35**, *R_f*: 0.42 (solvent B). The reaction mixture was treated with saturated sodium bicarbonate (11 mL). The residue from the solvent evaporation was dissolved in dichloromethane (500 mL) and then washed with water until pH neutral. The organic phase was dried (Na₂SO₄), filtered and evaporated. The residual oil was purified by flash chromatography on silica gel using solvent F as eluent to provide the title compound **37** (5.67 g, 94%) as a syrup, *R_f*: 0.33 (solvent F), [α]_D -80.1° (c 0.46, chloroform). ¹H-nmr (CDCl₃): δ 5.941 (d, 1H, *J*_{1,2} = 3.8 Hz, H-1), 5.901 (1H, H_c allyl) overlapping with H-1, 5.329 (1H, H_b allyl), 5.229 (1H, H_a allyl), 4.601 (d, 1H, *J*_{2,1} = 3.8 Hz, H-2), 4.159 (m, 2H, H_d and H_e allyl), 4.027 (d, 1H, *J*_{3,4} = 3.0 Hz, H-3), 3.727 (dd, 1H, *J*_{4,3} = 3.0 Hz, *J*_{4,5} = 7.2 Hz, H-4), 3.265 (ddd, 1H, *J*_{5,4} = 7.2 Hz, *J*_{5,6'} = 2.6 Hz, *J*_{5,6} = 3.8 Hz, H-5), 2.917 (dd, 1H, *J*_{6,6'} = 5.2 Hz, *J*_{6,5} = 3.8 Hz, H-6), 2.779 (dd, 1H, *J*_{6',6} = 5.2 Hz, *J*_{6',5} = 2.6 Hz; H-6'), 1.432, 1.263 (2s, each 3H, O₂C(CH₃)₂). ¹³C-nmr (CDCl₃): δ 133.90 (CH₂=CH-CH₂O), 117.63 (CH₂=CH-CH₂O), 111.91 (O₂C(CH₃)₂).

105.35 (C-1), 82.87, 81.91, 81.78 (all methine C-2, C-3, C-4), 71.41 (CH₂=CH-CH₂O), 48.13 (C-5), 46.97 (C-6), 26.85, 26.25 (O₂C(CH₃)₂).

Anal. calc. for C₁₂H₁₈O₅: C, 59.49; H, 7.49. Found: C, 59.55; H, 7.51.

3-O-Allyl-6-deoxy-1,2-O-isopropylidene- α -D-glucofuranose (38). - To a stirred solution of LiAlH₄ (2.21 g, 55.0 mmol) in dry diethyl ether (135 mL) was added dropwise a solution of compound **37** (5.48 g, 23.0 mmol) in diethyl ether (60 mL). Five minutes after the addition, tlc indicated the absence of starting material **37**. The reaction mixture was cooled to 0° and excess LiAlH₄ was decomposed by addition of ethyl acetate (6.5 mL) and stirring for 15 minutes. The mixture was then sequentially treated with methanol (16 mL) and water (107 mL). The removal of solvent gave a milky mixture which was extracted with dichloromethane (200 mL x 4). The combined dichloromethane extracts were dried (Na₂SO₄) and concentrated to a syrupy residue. Purification of product **38** was achieved by flash chromatography with solvent C as eluent. Pure **38** was obtained as a syrup (5.36 g, 97%); *R*_f: 0.46 (solvent C); [α]_D -61.7° (c 0.95, chloroform). ¹H-nmr (CDCl₃): δ 5.963 (d, 1H, *J*_{1,2} = 3.8 Hz, H-1), 5.914 (1H, H_c allyl) overlapping with H-1, 5.331 (1H, H_b allyl), 5.255 (1H, H_a allyl), 4.586 (d, 1H, *J*_{2,1} = 3.8 Hz, H-2), 4.213 (1H, H_d allyl), 4.114-3.971 (4H, H_e allyl overlapping with H-3 (δ 4.067, d, 1H, *J*_{3,4} = 3.3 Hz), H-4 (δ 3.971, dd, 1H, *J*_{4,3} = 3.3 Hz, *J*_{4,5} = 6.5 Hz, H-4), H-5 (δ 4.114, qdd, *J*_{5,6} = *J*_{5,4} = *J*_{5,OH} = 6.5 Hz, H-5)), 2.510 (d, 1H, *J*_{OH,5} = 6.7 Hz, OH), 1.501, 1.337 (2s, each 3H, O₂C(CH₃)₂) one overlapping with H-6, 1.325 (d, 3H, *J*_{6,5} = 6.5 Hz, H-6); ¹³C-nmr (CDCl₃): δ 133.49 (CH₂=CHCH₂O), 118.39 (CH₂=CHCH₂O), 111.58 (O₂C(CH₃)₂), 105.11 (C-1), 83.36, 82.39, 82.05, 65.87 (all methine C-2, 3,4,5), 70.85 (CH₂=CHCH₂O), 26.78, 26.27 (O₂C(CH₃)₂), 20.72 (C-6).

Anal. calc. for $C_{12}H_{20}O_5$: C, 59.00; H, 8.25. Found: C, 58.92; H, 8.08.

1,2,4-Tri-O-acetyl-3-O-allyl-6-deoxy- α,β -D-glucopyranose (40) and (41).

Aqueous 90% trifluoroacetic acid (13.18 mL) was added to a flask containing compound **38** (3.0 g, 12.30 mmol) at room temperature. After stirring for 35 minutes, tlc indicated the complete conversion of compound **38** (R_f : 0.47, solvent B) to more polar products (R_f : 0.03, solvent B or R_f : 0.39 and R_f : 0.35, solvent I), presumably the α and β anomers of compound **39**. Solvent was removed by evaporation and toluene was added and evaporated to give the α,β mixture **39** which was not characterized at this stage. The crude mixture of **39** was acetylated with acetic anhydride-pyridine (1:2, 45 mL) for 1 h at room temperature. Excess acetic anhydride was destroyed by the addition of ethanol (10 mL) to the ice-cold reaction mixture, followed by dichloromethane (150 mL) and water (125 mL). The aqueous layer was separated and extracted with dichloromethane. The combined dichloromethane extracts were washed sequentially with aqueous HCl (1 N, 50 mL x 2), saturated $NaHCO_3$ (100 mL) and water, then taken to dryness. Purification by flash chromatography (solvent L) provided the title compound **40** and **41** (2.79 g, 69%) as white solids (α/β :2/3).

Compound **40**, m.p. 68.5-70°C, R_f : 0.33 (solvent K); $[\alpha]_D +64.0^\circ$ (c 0.57, chloroform). ^1H -nmr ($CDCl_3$): δ 6.237 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1), 5.815 (1H, Hc allyl), 5.227 (1H, Hb allyl), 5.145 (1H, Ha allyl), 4.984 (dd, 1H, $J_{2,1} = 3.8$ Hz, $J_{2,3} = 9.5$ Hz, H-2), 4.839 (t, 1H, $J_{4,3} = J_{4,5} = 9.8$ Hz, H-4), 4.122 (m, 2H, Hd and Hc allyl), 3.905 (dq, 1H, $J_{5,4} = 10.0$ Hz, $J_{5,6} = 6.0$ Hz, H-5), 3.794 (t, 1H, $J_{3,4} = J_{3,2} = 9.7$ Hz, H-3), 2.125, 2.112, 2.055 (3s, each 3H, $OCOCH_3$), 1.177 (d, 3H, $J_{6,5} = 6.0$ Hz, H-6). ^{13}C -nmr ($CDCl_3$): δ 169.65, 169.53, 169.07

(3OCOCH₃), 134.59 (CH₂=CH-CH₂O), 116.63 (CH₂=CHCH₂O), 89.63, 74.42, 71.76, 68.33 (all methine C-1, 2,3,4,5), 73.43 (CH₂=CH-CH₂O), 20.96, 20.72 (3OCOCH₃), 17.42 (C-6).

Anal. calc. for C₁₅H₂₂O₈: C, 54.54; H, 6.71. Found: C, 54.66; H, 6.70.

Compound **41**, m.p. 101-102°C, *R*_f: 0.29 (solvent K), [α]_D +13.2° (c 0.54, chloroform). ¹H-nmr (CDCl₃): δ 5.777 (1H, H_c allyl), 5.624 (d, 1H, *J*_{1,2} = 8.5 Hz, H-1), 5.212 (1H, H_b allyl), 5.144 (1H, H_a allyl), 5.084 (dd, 1H, *J*_{2,1} = 8.5 Hz, *J*_{2,3} = 9.5 Hz, H-2), 4.848 (t, 1H, *J*_{4,3} = *J*_{4,5} = 9.5 Hz, H-4), 4.076 (m, 2H, H_d and H_e allyl), 3.596 (t, 1H, *J*_{3,4} = *J*_{3,2} = 9.5 Hz, H-3) overlapping with H-5, 3.600 (dq, 1H, *J*_{5,4} = 9.5 Hz, *J*_{5,6} = 6.0 Hz, H-5), 2.103, 2.100, 2.073 (3s, each 3H, OCOCH₃), 1.222 (d, 3H, *J*_{6,5} = 6.0 Hz, H-6). ¹³C-nmr (CDCl₃): δ 169.51, 169.44, 169.12 (all carbonyl O₂CCH₃); 134.27 (CH₂=CH-CH₂O), 117.05 (CH₂=CHCH₂O), 92.06, 79.82, 74.15, 71.94, 71.27 (all methine, C-1, 2,3,4,5), 72.94 (CH₂=CH-CH₂O), 20.96, 20.92, 20.83 (3O₂CCH₃), 17.35 (C-6).

Anal. calc. for C₁₅H₂₂O₈: C, 54.54; H, 6.71. Found: C, 54.61; H, 6.78.

p-Methoxybenzyl 2,4-di-O-acetyl-3-O-allyl-6-deoxy-α,β-D-glucopyranoside (**44**) and (**43**). - The mixture of compound **40** and **41** (1.02 g, 3.06 mmol) was dissolved in dichloromethane (12 mL) at 0°C and a solution of 45% hydrogen bromide in acetic acid (10 mL) was added at 0°C. The resulting solution was stirred for 15 minutes at 0°C when tlc showed the complete conversion of **40** and **41** (*R*_f: 0.38, solvent D) to a less polar product (*R*_f: 0.50, solvent D), presumably the glycosyl bromide **42**. The reaction mixture was diluted with cold dichloromethane (200 mL) and washed sequentially with saturated NaHCO₃, and with water. The organic layer was dried (Na₂SO₄) and concentrated. Then toluene (20 mL x 3) was

added to, and evaporated from, the pale yellow oily residue which was dissolved in toluene-nitromethane (1:1, 1 mL). This solution was added dropwise over 30 minutes to a stirred mixture of *p*-methoxybenzyl alcohol (625 μ L, 5.00 mmol), mercuric cyanide (927 mg, 6.0 mmol), 4 Å molecular sieves (2.5 g) and toluene-nitromethane (1:1, 6 mL) at room temperature under argon atmosphere. After 10 minutes, tlc indicated the absence of glycosyl bromide **42**. The reaction mixture was diluted with dichloromethane (50 mL) and filtered through Celite. The combined filtrate was washed with NaHCO₃ (3 x 60 mL), water (2 x 50 mL), dried (Na₂SO₄) and concentrated to a syrup which was purified by chromatography (solvent O).

Evaporation of early fractions furnished the α anomer **44** (68 mg, 7%), *R*_f 0.27 (solvent E); ¹H-nmr (CDCl₃): δ 7.257 (2H, aromatic), 6.883 (2H, aromatic), 5.807 (1H, H_c allyl), 5.203 (1H, H_b allyl), 5.110 (1H, H_a allyl), 5.027 (d, 1H, *J*_{1,2} = 4.0 Hz, H-1), 4.772 (2H, H-2, dd, *J*_{2,1} = 4.0 Hz, *J*_{2,3} = 9.8 Hz and H-4, t, *J*_{4,3} = 9.8 Hz, *J*_{4,5} = 10.0 Hz), 4.627 (d, 1H, *J*_{gem} = 12.0 Hz), 4.454 (d, 1H, *J*_{gem} = 12.0 Hz), 4.163, 4.047 (2H, H_d and H_e allyl), 3.836 (t, 1H, *J*_{3,4} = *J*_{3,2} = 9.8 Hz, H-3) overlapping with H-5 and C₆H₄OCH₃, 3.830 (qd, 1H, *J*_{5,4} = 10.0 Hz, *J*_{5,6} = 6.0 Hz, H-5), 3.811 (s, 3H, C₆H₄OCH₃), 2.083, 2.063 (2s, each 3H, OCOCH₃), 1.138 (d, 3H, *J*_{6,5} = 6.4 Hz, H-6).

Further elution provided a mixture of α and β anomers (50 mg) which were in the ratio of 1:5 (α / β) by ¹H-nmr.

Evaporation of the later fractions afforded the desired β -anomer, compound **43** (869 mg, 71%) as a white solid, m.p. 96-97°C, *R*_f 0.19 (solvent E); [α]_D -60.8° (*c* 0.5, chloroform). ¹H-nmr (CDCl₃): δ 7.211 (2H, aromatic), 6.870 (2H, aromatic), 5.760 (1H, H_c allyl), 5.830 (1H, H_b allyl), 5.110 (1H, H_a allyl), 5.018

(dd, 1H, $J_{2,1} = 8.0$ Hz, $J_{2,3} = 9.5$ Hz, H-2), 4.828 (t, 1H, $J_{4,3} = J_{4,5} = 9.0$ Hz, H-4), 4.800 (d, 1H, $J_{\text{gem}} = 12.0$ Hz, $\text{C}_6\text{H}_4\text{CH}_2\text{O}$) overlapping with H-4, 4.528 (d, 1H, $J_{\text{gem}} = 12.0$ Hz, $\text{C}_6\text{H}_4\text{CH}_2\text{O}$), 4.388 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.032 (2H, Hd and He allyl), 3.808 (s, 3H, $\text{C}_6\text{H}_4\text{OCH}_3$), 3.497 (t, 1H, $J_{3,2} = J_{3,4} = 9.5$ Hz, H-3), 3.426 (dq, 1H, $J_{5,4} = 9.5$ Hz, $J_{5,6} = 6.5$ Hz, H-5) slightly overlapped with H-3, 2.083, 2.040 (2s, 6H, 2OCOCH_3), 1.239 (d, 3H, $J_{6,5} = 6.5$ Hz, H-6). ^{13}C -nmr (CDCl_3): δ 169.5, 169.20 (2OCOCH_3), 159.38 (MeOC_6H_4), 134.52 ($\text{CH}_2=\text{CHCH}_2\text{O}$), 129.41 (C_6H_4), 129.31 ($\text{C}_6\text{H}_4\text{CH}_2\text{O}$), 116.79 ($\text{CH}_2=\text{CHCH}_2\text{O}$), 113.83 (C_6H_4), 99.14 (C-1), 79.92, 74.55, 72.81, 70.24 (all methine C-2, 3,4,5), 72.45 ($\text{CH}_2=\text{CHCH}_2\text{O}$), 69.93 ($\text{OCH}_2\text{C}_6\text{H}_4$), 55.23 ($\text{C}_6\text{H}_4\text{OCH}_3$), 20.99, 20.97 (2OCOCH_3), 17.48 (C-6).

Anal. calc. for $\text{C}_{21}\text{H}_{28}\text{O}_8$: C, 61.75; H, 6.91. Found: C, 61.63; H, 6.84.

p-Methoxybenzyl 3-O-allyl-2,4-di-O-benzyl-6-deoxy- β -D-glucopyranoside (46). - To a stirred solution of compound **43** (919 mg, 2.25 mmol) in dry methanol (6 mL) was added a methanolic solution of 0.5 N sodium methoxide (5 mL) at room temperature. After 3.5 h, tlc indicated the disappearance of compound **43** (R_f : 0.55, solvent B), the appearance of a more polar product (R_f : 0.32, solvent B), presumably compound **45**. The reaction mixture was neutralized with Amberlite IRC (50+) resin. The removal of resin by filtration and evaporation of the solvent provided the deacetylated product **45**, as a solid which was dissolved in *N,N*-dimethylformamide (20 mL). To this solution, sodium hydride that was prepared by washing a suspension of NaH (817 mg of a 50% dispersion in oil) with dry hexane (4 x 5 mL), was added and the mixture was stirred for 20 minutes at room temperature. Benzyl bromide (1 mL) was then added at 0°C under argon

atmosphere. Stirring was continued for 30 minutes at room temperature, by which time tlc indicated the completion of benzylation. The excess of NaH was destroyed by addition of methanol (3 mL) at 0°C. Solvent evaporation gave a solid residue which was purified by chromatography (solvent S) to provide **46** (1.09 g, 96%) as a white solid, m.p. 85.5-87°C, $[\alpha]_D -28.8^\circ$ (*c* 0.42, chloroform), *R*_f: 0.77 (solvent B); ¹H-nmr (CDCl₃): δ 7.351-7.273 (12H, aromatic), 6.855 (2H, aromatic), 5.955 (1H, H_c allyl), 5.259 (1H, H_b allyl), 5.149 (1H, H_a allyl), 4.912-4.850 (3H, C₆H₅CH₂O, C₆H₄CH₂O), 4.707-4.545 (3H, benzylic), 4.433 (d, 1H, *J*_{1,2} = 7.8 Hz, H-1), 4.384 (dddd, 1H, CH₂=CHCH₂O) partially overlapped with H-1, 4.262 (dddd, 1H, CH₂=CHCH₂O), 3.803 (s, 3H, C₆H₄OCH₃), 3.452 (t, 1H, *J*_{3,2} = *J*_{3,4} = 8.5 Hz, H-3), 3.400 (dd, 1H, *J*_{2,1} = 7.8 Hz, *J*_{2,3} = 8.5 Hz, H-2) partially overlapped with H-5, H-3, 3.356 (qd, 1H, *J*_{5,4} = 9.0 Hz, *J*_{5,6} = 6.0 Hz, H-5), 3.141 (dd, 1H, *J*_{4,3} = 8.5 Hz, *J*_{4,5} = 9.0 Hz, H-4), 1.323 (d, 3H, *J*_{6,5} = 6.0 Hz, H-6). ¹³C-nmr (CDCl₃): δ 159.38 (CH₃OC₆H₄), 138.64, 138.33 (OCH₂C₆H₄, OCH₂C₆H₅), 135.30 (CH₂=CHCH₂O), 129.69 (C₆H₄CH₂O), 128.48, 128.34, 128.18, 127.87, 127.64 (C₆H₅CH₂O), 116.74 (CH₂=CHCH₂O), 113.86 (C₆H₄CH₂O), 102.21 (C-1), 84.33, 83.38, 82.57, 71.14 (all methine C-2, 3,4,5), 75.35, 74.69, 74.51 (C₆H₄CH₂O, C₆H₅CH₂O), 70.96 (CH₂=CH-CH₂O), 55.32 (C₆H₄OCH₃), 17.97 (C-6).

Anal. calc. for C₃₁H₃₆O₆: C, 73.79; H, 7.19. Found: C, 73.89; H, 7.22.

p-Methoxybenzyl 2,4-di-O-benzyl-6-deoxy-β-D-glucopyranoside (**47**). - A solution of compound **46** (800 mg, 1.59 mmol), tris(triphenylphosphine)rhodium(I)chloride (104 mg, 0.11 mmol), and 1,8 diazobicyclo[2.2.2]octane (46.8 mg, 0.43 mmol) in ethanol-toluene-water (7:3:1; 78 mL) was heated at reflux overnight.

After solvent evaporation, the residue was dissolved in acetone (80 mL) containing mercuric oxide (24 mg). To this solution was added a solution of mercuric chloride (3.31 g) in acetone-water (9:1, 40 mL), and the mixture was stirred at room temperature for 45 minutes. After evaporation of the solvent, the residue was taken up in dichloromethane (200 mL) and washed with 30% aqueous KBr followed by water. Drying (Na_2SO_4) and evaporation left a solid residue that was purified by chromatography using solvent P as eluent. Pure **47** was obtained (644 mg, 88%) as a solid; R_f : 0.35 (solvent E). $[\alpha]_D -11.8^\circ$ (c 0.45, chloroform), m.p. 28-30°C; ^1H -nmr (CDCl_3): δ 7.320 (12H, aromatic), 6.875 (2H, C_6H_4 aromatic), 4.972-4.853 (m, 3H, $3J_{\text{gem}} = 11.0$ Hz), 4.684-4.551 (m, 3H, $3J_{\text{gem}} = 11.0$ Hz), 4.457 (d, 1H, $J_{1,2} = 7.5$ Hz, H-1), 3.674 (ddd, 1H, $J_{3,2} = 9.0$ Hz, $J_{3,4} = 9.0$ Hz, $J_{3,\text{OH}} = 2.0$ Hz, H-3), 3.400 (qd, 1H, $J_{5,4} = 9.0$ Hz, $J_{5,6} = 6.0$ Hz, H-5), 3.291 (dd, 1H, $J_{2,1} = 7.5$ Hz, $J_{2,3} = 9.0$ Hz, H-2), 3.134 (t, 1H, $J_{4,3} = J_{4,5} = 9.0$ Hz, H-4), 2.429 (d, 1H, $J_{\text{OH},3} = 2.0$ Hz, OH), 1.343 (d, 3H, $J_{6,5} = 6.0$ Hz, H-6); ^{13}C -nmr (CDCl_3): δ 159.41 ($\text{C}_6\text{H}_4\text{OCH}_3$), 138.47 ($\text{C}_6\text{H}_5\text{CH}_2\text{O}$), 129.53 ($\text{OCH}_2\text{C}_6\text{H}_4$), 129.71, 113.88 ($\text{C}_6\text{H}_4\text{CH}_2\text{O}$), 128.54, 128.47, 128.15, 128.07, 127.88, 127.82 ($\text{C}_6\text{H}_5\text{CH}_2\text{O}$), 101.79 (C-1), 82.99, 81.68, 71.16 (all methine C-2, 3,4,5), 74.81, 74.43 ($\text{C}_6\text{H}_5\text{CH}_2\text{O}$), 70.86 ($\text{C}_6\text{H}_4\text{CH}_2\text{O}$), 55.31 ($\text{C}_6\text{H}_4\text{OCH}_3$), 18.06 (C-6).

Anal. calc. for $\text{C}_{28}\text{H}_{32}\text{O}_6$: C, 72.39; H, 6.94. Found: C, 71.95; H, 6.85.

p-Methoxybenzyl 3-O-(2,4-di-O-acetyl-3-O-allyl-6-deoxy- β -D-glucopyranosyl)-2,4-di-O-benzyl-6-deoxy- α - and β -D-glucopyranoside (**48**, α) and (**49**, β). A mixture of alcohol **47** (414 mg, 0.89 mmol), mercuric cyanide (378 mg, 1.50 mmol) and 4 Å molecular sieves (1.66 g) in dry toluene-nitromethane (1:1, 4 mL)

was stirred at 32°C for 10 minutes. A solution of glycosyl bromide **42** (1.50 mmol) in toluene-nitromethane (1:1, 1 mL) was then added dropwise over 15 minutes. After stirring for 5 minutes, tlc showed the absence of glycosyl bromide **42** (R_f : 0.54, solvent E) and the presence of unreacted alcohol **47** ($\approx 30\%$), R_f : 0.42 (solvent E). Since the coupling products **48** and **49** (R_f : 0.30, solvent E) were sensitive to the reaction conditions and decomposed to more polar products, the reaction mixture was immediately removed from the bath at 32°C. The reaction mixture was diluted with dichloromethane (25 mL). The solids were removed by filtration and washed exhaustively with dichloromethane. The combined filtrate was washed with saturated NaHCO_3 (3 x 40 mL), water (2 x 50 mL) and dried (Na_2SO_4). Solvent evaporation left a syrup which was subjected to chromatography using solvent F for elution.

Evaporation of the early fractions yielded recovered alcohol **47** (106 mg, 26%). Further elution provided a mixture of α and β anomers **48** and **49** (475 mg, 73%) in the ratio of α/β : 1/10. The isolation of the β anomer, compound **49** (432 mg, 66%), m.p. 79-80°C, was performed using a second silica gel column. $[\alpha]_D -23.4^\circ$ (c 0.24, chloroform). ^1H -nmr (CDCl_3): δ 7.420-7.250 (12H, aromatic), 6.862 (2H, aromatic), 5.784 (1H, H_c allyl), 5.221 (1H, H_b allyl), 5.149 (1H, H_a allyl), 5.034-5.000 (3H, H-1', H-2', benzylic), 4.961 (d, 1H, $J_{\text{gem}} = 10.0$ Hz), 4.868 (d, 1H, $J_{\text{gem}} = 11.0$ Hz) partially overlapped with H-4', 4.822 (t, 1H, $J_{4',5'} = J_{4',3'} = 9.5$ Hz, H-4'), 4.586-4.512 (3d, each 1H, $J_{\text{gem}} = 11.6$ Hz, $2J_{\text{gem}} = 12.0$ Hz), 4.415 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.054 (2H, H_d and H_e allyl) unresolved, 3.932 (t, 1H, $J_{3,4} = J_{3,2} = 9.0$ Hz, H-3), 3.805 (s, 3H, $\text{C}_6\text{H}_4\text{OCH}_3$), 3.513-3.273 (4H, H-3', H-5') overlapping with H-2 (δ 3.373, dd, 1H, $J_{2,3} = 8.5$ Hz, $J_{2,1} = 8.0$ Hz) and H-5 (δ 3.286, qd, 1H, $J_{5,4} = 9.0$ Hz, $J_{5,6}$

= 6.2 Hz), 3.120 (t, 1H, $J_{4,5} = J_{4,3} = 9.0$ Hz, H-4), 2.114 (s, 3H, OCOCH₃), 2.084 (s, 3H, OCOCH₃), 1.317 (d, 3H, $J_{6',5'} = 6.2$ Hz, H-6'), 1.124 (d, 3H, $J_{6,5} = 6.2$ Hz, H-6). ¹³C-nmr (CDCl₃): δ 169.62, 169.09 (all carbonyl carbon), 138.41 (C₆H₅CH₂O), 134.54 ((CH₂=CH-CH₂O), 129.88 (C₆H₄OCH₃), 129.49 (C₆H₄CH₂O), 128.73, 128.49, 128.34, 128.25, 127.92, 127.73 (C₆H₅), 118.88 (CH₂=CHCH₂O), 113.90 (C₆H₄), 101.77, 100.23 (C-1, 1'), 83.45, 81.30, 80.73, 79.98, 74.79, 73.32, 71.06, 70.08 (all methine C-2, 2', 3, 3', 4, 4', 5, 5'), 75.23, 74.75, 72.48 (all benzylic carbon), 70.92 (CH₂=CHCH₂O), 55.33 (C₆H₄OCH₃), 21.15, 21.02 (2OCOCH₃), 18.04, 17.39 (C-6, 6').

Anal. calc. for C₄₁H₅₀O₁₂: C, 67.01; H, 6.86. Found: C, 67.11; H, 6.61.

p-Methoxybenzyl 3-O-(3-O-allyl-2,4-di-O-benzyl-6-deoxy-β-D-glucopyranosyl)-2,4-di-O-benzyl-6-deoxy-β-D-glucopyranoside (**51**). - Compound **49** (1.04 g, 1.42 mmol) was dissolved in dry methanol (12 mL). To this solution was added a solution of 0.5 N sodium methoxide in methanol (8 mL) and the mixture was stirred for 3 h. Tlc indicated the complete conversion of compound **49** (*R*_f: 0.58, solvent B) to a more polar product (*R*_f: 0.42, solvent B), presumably the deacetylated product **50**. After neutralization with Amberlite IRC-50 (H⁺) and removal of the resin by filtration, solvent evaporation left a solid residue, compound **50**. This material was not characterized at this stage but was benzylated directly with sodium hydride (1.07 g, 50% dispersion in oil) and benzyl bromide (1.40 mL, 11.77 mmol) in *N,N*-dimethylformamide (15 mL) for 1.5 h at room temperature. Excess NaH was then destroyed by addition of methanol at 0°C. After solvent evaporation, the residue was purified by chromatography on a silica gel column using solvent T as eluent to provide the desired compound **51** (1.12 g, 95%) as a solid, *R*_f: 0.36

(solvent U); $[\alpha]_D +4.5^\circ$ (c 0.78, chloroform). ^1H -nmr (CDCl_3): δ 7.432-7.260 (22H, aromatic), 6.854 (2H, aromatic), 5.992 (1H, Hc allyl), 5.300 (1H, Hb allyl), 5.181 (1H, Ha allyl), 5.053-4.992 (2H, $J_{\text{gem}} = 10.5$ Hz, $J_{\text{gem}} = 11.5$ Hz), 4.969 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1'), 4.887-4.486 (8H, benzylic protons), 4.468-4.291 (m, 3H, Hd, He allyl and H-1 (δ 4.424, d, 1H, $J_{1,2} = 8.0$ Hz, H-1)), 4.013 (t, 1H, $J_{3,4} = J_{3,2} = 9.0$ Hz, H-3), 3.799 (s, 3H, $\text{C}_6\text{H}_4\text{OCH}_3$), 3.488-3.310 (4H, H-5 overlapping with H-2, H-4', H-2'; δ 3.459, dd, 1H, $J_{2,3} = 9.0$ Hz, $J_{2,1} = 8.0$ Hz, H-2), 3.395, t, 1H, $J_{4',3'} = J_{4',5'} = 8.8$ Hz, H-4'; 3.338, dd, 1H, $J_{2',1'} = 8.0$ Hz, $J_{2',3'} = 8.8$ Hz, H-2'), 3.260-3.082 (3H, H-5', H-4, H-3'; δ : 3.217, qd, 1H, $J_{5',4'} = 9.5$ Hz, $J_{5',6'} = 6.0$ Hz, H-5'; 3.155, t, 1H, $J_{4,5} = 9.5$ Hz, $J_{4,3} = 9.0$ Hz, H-4; 3.117, t, 1H, $J_{3',4'} = 9.0$ Hz, $J_{3',2'} = 9.0$ Hz, H-3'), 1.352 (d, 3H, $J_{6,5} = 6.0$ Hz, H-6), 1.220 (d, 3H, $J_{6',5'} = 6.0$ Hz, H-6'). ^{13}C -nmr (CDCl_3): δ 159.43 ($\text{C}_6\text{H}_4\text{OCH}_3$), 138.76, 138.60, 138.30, 138.27 ($\text{C}_6\text{H}_5\text{CH}_2\text{O}$), 135.35 ($\text{CH}_2=\text{CHCH}_2\text{O}$), 129.82 ($\text{C}_6\text{H}_4\text{CH}_2\text{O}$), 129.55 (C_6H_4), 128.74, 128.70, 128.50, 128.29, 128.21, 128.07, 127.91, 127.72, 127.55 ($4\text{C}_6\text{H}_5$), 116.68 ($\text{CH}_2=\text{CHCH}_2\text{O}$), 113.89 (C_6H_4), 102.34, 101.95 (C-1, 1'), 84.39, 83.89, 83.49, 83.23, 81.76, 80.15, 70.94, 70.84 (all methine C-2, 2', 3, 3', 4, 4', 5, 5'), 75.41, 75.04, 74.93, 74.76, 74.48 (all benzylic carbon), 71.00 ($\text{CH}_2=\text{CHCH}_2\text{O}$), 55.31 ($\text{C}_6\text{H}_4\text{OCH}_3$), 18.11, 17.97 (C-6, 6').

Anal. calc. for $\text{C}_{51}\text{H}_{58}\text{O}_{10}$: C, 73.71; H, 7.04. Found: C, 73.87; H, 6.75.

p-Methoxybenzyl 2,4-di-O-benzyl-6-deoxy-3-O-[2,4-di-O-benzyl-6-deoxy-3-O-(2-hydroxyethyl)- β -D-glucopyranosyl]- β -D-glucopyranoside (53). - Compound 51 (512 mg, 61.6 mmol) was dissolved in dry methanol-dichloromethane (1:1, 6 mL) and ozonized by passing the O_3 gas through for 4

minutes at -78°C . Tlc indicated the absence of allyl compound **51** (R_f : 0.79, solvent B) and the reaction mixture had a blue colour. The colour disappeared after addition of dimethyl sulfide (1.2 mL) at -78°C . After stirring for 0.5 h at room temperature, the product **52** was obtained (R_f : 0.59, solvent B) and its aldehyde structure was confirmed from the ^1H -nmr spectrum (δ 9.618, t, $J = 0.9$ Hz). This aldehyde was not further characterized but reduced directly to alcohol using sodium borohydride (49.55 mg, 1.31 mmol) for 15 minutes. After neutralization with Amberlite IRC 50 (H^+) resin, solvent was removed by evaporation leaving an oily residue which was purified by chromatography using solvent O as eluent, to provide the desired compound **53** (431.7 mg, 82%) as a syrup; $[\alpha]_D -1.5^{\circ}$ (c 1.3, chloroform) R_f : 0.53, solvent B. ^1H -nmr (CDCl_3): δ 7.442-7.238 (22H, aromatic), 6.862 (2H, aromatic), 5.063-5.013 (2H, benzylic protons) 4.986 (d, 1H, $J_{1'2'} = 7.5$ Hz, H-1'), 4.891-4.782 (4H, benzylic protons), 4.651-4.476 (4H, benzylic protons), 4.432 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.035 (t, 1H, $J_{3,4} = J_{3,2} = 9.0$ Hz, H-3), 3.912 (2H, $\text{OCH}_2\text{CH}_2\text{O}$), 3.800 (s, 3H, $\text{C}_6\text{H}_4\text{OCH}_3$), 3.671 (2H, $\text{OCH}_2\text{CH}_2\text{O}$), 3.482 (dd, 1H, $J_{2,1} = 8.0$ Hz, $J_{2,3} = 9.0$ Hz, H-2) overlapping with H-3', 3.468 (t, 1H, $J_{3',2'} = J_{3',4'} = 9.2$ Hz, H-3') overlapping with H-5', 3.394 (qd, 1H, $J_{5',6'} = 6.0$ Hz, $J_{5',4'} = 9.0$ Hz, H-5'), overlapping with H-2', 3.329 (dd, 1H, $J_{2',1'} = 7.6$ Hz, $J_{2',3'} = 9.2$ Hz, H-2'), 3.210 (qd, 1H, $J_{5,6} = 6.0$ Hz $J_{4,5} = 9.4$ Hz, H-5) overlapping with H-4, 3.167 (t, 1H, $J_{4,3} = J_{4,5} = 9.0$ Hz, H-4), 3.100 (t, 1H, $J_{4',3'} = J_{4',5'} = 9.0$ Hz, H-4'), 2.940 (t, 1H, $J_{\text{OH},\text{CH}_2\text{OH}} = 6.5$ Hz, OH), 1.361, 1.246 (2d, each 3H, $J_{6',5'} = J_{6,5} = 6.0$ Hz, H-6, H-6'). ^{13}C -nmr (CDCl_3): δ 159.45 ($\text{C}_6\text{H}_4\text{OCH}_3$), 138.57, 138.13, 137.98, 137.81 ($4\text{C}_6\text{H}_5\text{CH}_2\text{O}$), 129.84 (C_6H_4), 129.47 ($\text{C}_6\text{H}_4\text{CH}_2\text{O}$), 128.70, 128.62, 128.57, 128.45, 128.43, 128.40, 128.22, 128.18, 128.06, 127.95, 127.85, 127.73

(4C₆H₅), 113.89 (C₆H₄CH₂O), 102.33, 101.89 (C-1, C-1'), 84.59, 84.43, 83.46, 82.65, 81.61, 80.00, 70.90 (all methine C-2, 2',3,3',4,4',5,5'), 75.48, 75.15, 74.93, 74.84, 74.75 (4C₆H₅CH₂O, C₆H₄CH₂O), 70.97 (OCH₂CH₂OH), 62.91 (OCH₂CH₂OH), 55.30 (C₆H₄OCH₃), 18.11, 17.92 (C-6, 6').

Anal. calc. for C₅₀H₅₈O₁₁: C, 71.92; H, 7.00. Found: C, 72.00; H, 6.63.

p-Methoxybenzyl 2,4-di-O-benzyl-6-deoxy-3-O-[2,4-di-O-benzyl-3-O-(4-carboxy-3-oxabutyl)-6-deoxy- β -D-glucopyranosyl]- β -D-glucopyranoside (**54**). - A solution of compound **53** (391.7 mg, 0.50 mmol) in dry tetrahydrofuran was cooled to -78°C. To this stirring solution was added a solution of *n*-BuLi (2.5 M in hexane, 225 μ L, 0.56 mmol) and the reaction mixture was stirred for 15 minutes at -78°C. An addition of bromoacetic acid lithium salt (Br-CH₂COOLi, 136.2 mg, 0.94 mmol) was made to the pale yellow solution. Stirring was then continued for an additional 10 minutes at room temperature and for 2.5 h at 67°C (refluxing temperature) by which time tlc indicated the presence of only little starting alcohol **53** ($\approx$ 5%), *R*_f: 0.43 (solvent B) and a more polar compound (*R*_f: 0.04, solvent B), presumably the acid product **54**. The residue obtained after solvent evaporation was dissolved in methanol and cooled to -5°C. Neutralization of the mixture with Amberlite 120 (H⁺) at -5°C, subsequent removal of resin by filtration and evaporation left a slightly yellow residue which was subjected to chromatography on a silica gel column using solvent V as eluent. Pure **54** was obtained (360.4 mg, 86%) as a syrup (*R*_f: 0.30, solvent V). [α]_D +2.5° (*c* 0.40, chloroform). ¹H-nmr (CDCl₃): δ 7.422-7.240 (22H, aromatic), 6.842 (2H, aromatic), 5.033 (2H, *J*_{gem} = 10.0 Hz, *J*_{gem} = 11.0 Hz, 4.974 (d, 1H, *J*_{1',2'} = 7.5 Hz, H-1'), 4.877-4.757 (4H, benzylic proton), 4.657-4.490 (4H, benzylic proton), 4.423 (d, 1H, *J*_{1,2} =

8.0 Hz, H-1), 4.068-3.944 (4H, $\text{OCH}_2\text{CH}_2\text{OCH}_2\text{COOH}$), 3.793 (s, 3H, $\text{C}_6\text{H}_4\text{OCH}_3$), 3.598 (2H, $\text{OCH}_2\text{CH}_2\text{O}$) unresolved, 3.467 (dd, 1H, $J_{2,3} = 9.0$ Hz, $J_{2,1} = 8.0$ Hz, H-2) partially overlapped with H-5', 3.433-3.030 (3H, H-5', H-3' (δ 3.371, dd, 1H, $J_{3',2'} = 8.5$ Hz, $J_{3',4'} = 9.0$ Hz), H-2' (δ 3.338, dd, 1H, $J_{2',3'} = J_{2',3'} = J_{2',1'} = 8.2$ Hz)), 3.208 (qd, 1H, $J_{5,4} = 9.0$ Hz, $J_{5,6} = 6.1$ Hz, H-5) overlapping with H-4, 3.168 (t, 1H, $J_{4,3} = J_{4,5} = 9.0$ Hz, H-4), 3.124 (dd, 1H, $J_{4',3'} = 8.5$ Hz, $J_{4',5'} = 9.0$ Hz, H-4') partially overlapped with H-4, 1.358 (d, 3H, $J_{6',5'} = 6.2$ Hz, H-6'), 1.239 (d, 3H, $J_{6,5} = 6.0$ Hz, H-6). ^{13}C -nmr (CDCl_3): δ 172.15 (CH_2COOH), 159.41 ($\text{C}_6\text{H}_4\text{OCH}_3$), 138.52, 138.36, 138.02 ($4\text{C}_6\text{H}_5\text{CH}_2\text{O}$), 129.81 ($\text{OCH}_2\text{C}_6\text{H}_4$), 129.47 (C_6H_4), 128.63, 128.52, 128.50, 128.35, 128.27, 128.21, 128.13, 128.08, 127.98, 127.68 ($4\text{C}_6\text{H}_5$), 113.86 (C_6H_4), 102.35, 101.87 (C-1, C-1'), 85.26, 83.83, 83.49, 82.80, 81.76, 80.30, 70.88 (all methine C-2, 2', 3, 3', 4, 4', 5, 5'), 75.35, 74.97, 74.65 ($4\text{C}_6\text{H}_5\text{CH}_2\text{O}$), 72.21 ($\text{C}_6\text{H}_4\text{CH}_2\text{O}$), 71.95 (OCH_2COOH), 70.96 ($\text{OCH}_2\text{CH}_2\text{O}$), 68.61 ($\text{CH}_2\text{OCH}_2\text{COOH}$), 55.28 ($\text{C}_6\text{H}_4\text{OCH}_3$), 18.10, 17.94 (C-6, C-6').

Anal. calc. for $\text{C}_{52}\text{H}_{60}\text{O}_{13}$: C, 69.94; H, 6.71. Found: C, 70.01; H, 6.75.

p-Methoxybenzyl 2,4-di-O-benzyl-6-deoxy-3-O-[2,4-di-O-benzyl-6-deoxy-3-O-(4-methoxycarbonyl-3-oxabutyl)- β -D-glucopyranosyl]- β -D-glucopyranoside (59). - A solution of compound 54 (168 mg, 0.19 mmol) in dichloromethane (2 mL) was cooled at 0°C and to this was added dropwise an ethereal solution of diazomethane (0.1 N) until the yellow colour of diazomethane persisted. The reaction mixture was left at 0°C for another 20 minutes, by which time tlc indicated the incomplete conversion of compound 54 (R_f : 0.97, solvent N). More

diazomethane was added and the reaction was left at 0°C for an additional 15 minutes but no further change was observed on the tlc plate. Excess diazomethane was destroyed by dropwise addition of acetic acid at 0°C. Solvent evaporation left a syrup residue, the purification of which was achieved by flash chromatography using solvent E as eluent. The title compound **59** was obtained as a syrup (140 mg, 82%), *R*_f: 0.21 (solvent E), [α]_D + 2.8° (*c* 0.51, chloroform). ¹H-nmr (CDCl₃): δ 7.970–7.282 (22H, aromatic), 6.913 (2H, aromatic C₆H₄OCH₃), 5.113–5.056 (2H, *J*_{gem} = 10.5 Hz, *J*_{gem} = 11.5 Hz), 5.028 (m, 1H, *J*_{1',2'} = 8.0 Hz, H-1', virtually coupled to H-3) partially overlapped with benzylic proton, 4.993 (d, 1H, *J*_{gem} = 10.5 Hz), 4.938–4.881 (2d, each 1H, *J*_{gem} = 11.5 Hz, *J*_{gem} = 11.2 Hz), 4.810 (d, 1H, *J*_{gem} = 10.0 Hz), 4.681 (d, 1H, *J*_{gem} = 10.5 Hz), 4.610 (d, 2H, *J*_{gem} = 10.5 Hz), 4.559 (d, 1H, *J*_{gem} = 10.0 Hz), 4.468 (d, 1H, *J*_{1,2} = 8.0 Hz, H-1), 4.212–4.021 (5H, OCH₂CH₂O, OCH₂COOCH₃ (δ 4.157, 2s), H-3 (δ 4.083, t, 1H, *J*_{3,2} = *J*_{3,4} = 9.0 Hz)), 3.842 (s, 3H, C₆H₄OCH₃), 3.768 (m, 2H, OCH₂CH₂O) partially overlapped with COOCH₃, 3.743 (s, 3H, COOCH₃), 3.518 (dd, 1H, *J*_{2,3} = 9.0 Hz, *J*_{2,1} = 8.0 Hz, H-2), 3.441 (qd, 1H, *J*_{5',6'} = 6.0 Hz, H-5') partially overlapped with H-2, H-2' and H-3', 3.461–3.387 (m, 2H, H-2', H-3'), 3.280 (qd, 1H, *J*_{5,4} = 9.0 Hz, *J*_{5,6} = 6.0 Hz, H-5) overlapping with H-4, H-4', 3.253–3.164 (m, 2H, H-4 (δ 3.215, t, 1H, *J*_{4,5} = *J*_{4,3} = 9.0 Hz) and H-4'), 1.418 (d, 3H, *J*_{6',5'} = 6.0 Hz, H-6'), 1.285 (d, 3H, *J*_{6,5} = 6.0 Hz, H-6). ¹³C-nmr (CDCl₃): δ 170.79 (COOCH₃), 159.42 (C₆H₄OCH₃), 138.75, 138.58, 138.36, 138.31 (4C₆H₅CH₂O) 129.82 (C₆H₄CH₂O), 129.53 (C₆H₄), 128.67, 128.49, 128.30, 128.21, 128.09, 127.87, 127.72, 127.57 (4C₆H₅), 113.88 (C₆H₄), 102.30, 101.94 (C-1, C-1'), 85.54, 87.73, 83.50, 83.10, 81.76, 80.08, 70.94, 70.82 (all methine, C-2, 2', 3, 3', 4, 4', 5, 5'), 75.30, 74.98, 74.76

(4C₆H₅CH₂O), 72.75 (C₆H₄CH₂O), 71.29 (OCH₂COOCH₃), 71.00 (OCH₂CH₂OCH₂), 68.59 (CH₂OCH₂COOCH₃), 51.73 (COOCH₃) 18.11, 17.96 (C-6, C-6').

Anal. calc. for C₅₃H₆₂O₁₃: C, 70.18; H, 6.89. Found: C, 70.28; H, 6.74.

2,4-Di-O-benzyl-6-deoxy-3-O-[2,4-di-O-benzyl-6-deoxy-3-O-(4-methoxycarbonyl-3-oxabutyl)-β-D-glucopyranosyl]-β-D-glucopyranose (56). - A solution of compound **54** (101 mg, 0.11 mmol) in 80% aqueous acetic acid (2.9 mL) was stirred at 102°C for 12 h. To monitor the reaction by tlc, *p*-anisidine phthalate solution (1 M) that only gives a positive test on reducing sugar was used for detection. The reaction was terminated when tlc indicated, beside the main reaction product, the formation of new polar side products (*R_f*: 0.14, solvent V) although compound **54** (≈ 20%, *R_f*: 0.51, solvent V) was not completely consumed. Aqueous acetic acid was evaporated at 35°C and toluene (5 mL x 5) was added and then evaporated leaving a syrup residue which was filtered through a short column of silica gel using solvent V for elution.

The reducing acid disaccharides **55** thus obtained were not characterized, but esterified directly with an excess amount of ethereal diazomethane (0.1 N) as described for the preparation of compound **59**. Solvent evaporation followed by chromatographic purification (solvent D) of the residue gave compound **56** (57 mg, 81%) as a syrup, *R_f*: 0.22 (solvent D). Compound **54** (21 mg, 21%) was also recovered. ¹H-nmr (CDCl₃): δ 7.450-7.200 (m, 20H, aromatic), 2.855 (d, 1H of α or β anomer, *J*_{OH,1} = 2.5 Hz, OH), 1.333-1.230 (m, 6H, *J*_{6,5} = *J*_{6',5'} ≈ 6.0 Hz, H-6, H-6'). ¹³C-nmr (CDCl₃): δ 171.59 (COOCH₃), 139.72, 139.58, 139.40, 139.29, 139.17, 139.14, 139.05, 138.50 (4C₆H₅CH₂O), 129.44,

129.37, 129.29, 129.23, 129.19, 129.13, 129.03, 128.89, 128.82, 128.73, 128.66, 128.55, 128.48, 128.37 (C_6H_5), 103.02, 97.90, 91.52, 86.38, 86.35, 85.37, 84.55, 84.51, 84.09, 83.96, 82.56, 82.47, 82.38, 80.72, 71.94, 71.69, 67.23 (all methine (C-1, 1', 2, 2', 3, 3', 4, 4', 5, 5')), 76.07, 75.83, 75.76, 75.56, 74.10 ($C_6H_5CH_2O$), 73.54 (CH_2COOCH_3), 72.08 (OCH_2CH_2O), 69.36 ($CH_2OCH_2COOCH_3$), 52.54 ($COOCH_3$), 18.91, 18.85, 18.77 (C-6, C-6').

Methyl 2,4-di-O-benzyl-6-deoxy-3-O-[2,4-di-O-benzyl-6-deoxy-3-O-(4-methoxy-carbonyl-3-oxabutyl)- β -D-glucopyranosyl]- α,β -D-glucopyranoside (58, α) and (57, β). - Methyl iodide (19 μ L, 0.29 mmol) was added at 0°C under argon atmosphere to a mixture of compound **56** (46 mg, 5.85×10^{-2} mmol) and silver oxide (70 mg, 0.29 mmol) in toluene (1 mL). The reaction mixture was then stirred for 67 h at room temperature. The tlc of the reaction mixture revealed the presence of a major product ($\approx 85\%$, R_f : 0.35, solvent E), presumably the β -glycoside **57**, and the minor product **58** ($\approx 10\%$, R_f : 0.24, solvent E). Solids were removed by filtration and washed with dichloromethane. Removal of the solvent gave a colourless syrup residue which was purified by column chromatography using solvent E for elution.

Evaporation of early fractions furnished the β -anomer **57** (34 mg, 73%) as a syrup; $[\alpha]_D + 27.8^\circ$ (c 0.55, chloroform). ^1H -nmr ($CDCl_3$): δ 7.450-7.236 (20H, aromatic), 5.049 (d, 1H, $J_{gem} = 10.5$ Hz), 5.012 (d, 1H, $J_{gem} = 11.5$ Hz), 4.990-4.962 (m, 1H, H-1', virtually coupled to H-3) partially overlapped with one benzylic proton, 4.9348 (d, 1H, $J_{gem} = 10.5$ Hz), 4.847 (d, 1H, $J_{gem} = 11.5$ Hz), 4.723 (d, 1H, $J_{gem} = 10.0$ Hz), 4.628 (d, 1H, $J_{gem} = 10.5$ Hz), 4.526 (t, 2H, $J_{gem} = 10.5$ Hz), 4.227 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1) 4.158-3.965 (5H,

OCH₂CH₂O, OCH₂COOCH₃ (δ 4.100, 2s, 2H), H-3 (δ 4.024, t, 1H, $J_{3,4} = J_{3,4} = 9.0$ Hz)), 3.715 (2H, OCH₂CH₂O) partially overlapped with COOCH₃, 3.696 (s, 3H, COOCH₃), 3.546 (s, 3H, OCH₃), 3.429-3.336 (m, 4H, H-5', H-2', H-2, H-3'), 3.282-3.130 (m, 3H, H-5, H-4', H-4 (δ 3.129, t, 1H, $J_{4,3} = J_{4,5} = 9.0$ Hz)), 1.338 (d, 3H, $J_{6',5'} = 6.0$ Hz, H-6'), 1.238 (d, 3H, $J_{6,5} = 6.0$ Hz, H-6). ¹³C-nmr (CDCl₃): δ 170.77 (COOCH₃), 138.77, 138.53, 138.39, 138.35 (4C₆H₅CH₂O), 128.66, 128.57, 128.47, 128.34, 128.28, 128.26, 128.20, 128.10, 127.84, 127.72, 127.56 (4C₆H₅), 104.32, 102.28 (C-1, C-1'), 85.54, 83.71, 83.60, 83.10, 81.75, 80.00, 70.89, 70.84 (all methine C-2, 2', 3, 3', 4, 4', 5, 5'), 75.27, 74.98, 74.73 (4C₆H₅CH₂O), 72.73 CH₂COOCH₃), 71.27 (OCH₂CH₂O), 68.56 (CH₂OCH₂COOCH₃), 57.11 (OCH₃), 51.71 (COOCH₃), 18.04, 17.96 (C-6, C-6').

Anal. calc. for C₄₆H₅₆O₁₂: C, 68.98; H, 7.05. Found: C, 69.02; H, 7.08.

Further elution provided α -anomer **58** (4.5 mg, 10%) as a syrup. ¹H-nmr (CDCl₃): δ 5.043-4.996 (2d, each 1H, $J_{\text{gem}} = 10.2$ Hz, $J_{\text{gem}} = 11.0$ Hz), 4.984 (d, 1H, $J_{1',2'} = 7.2$ Hz, H-1') partially overlapped with a benzylic proton, 4.933 (d, 1H, $J_{\text{gem}} = 11.0$ Hz), 4.881 (d, 1H, $J_{\text{gem}} = 11.0$ Hz), 4.635 (d, 1H, $J_{\text{gem}} = 11.0$ Hz), 4.553 (d, 1H, $J_{\text{gem}} = 11.0$ Hz), 4.371 (d, 1H, $J_{1,2} = 4.0$ Hz, H-1) overlapping with a benzylic proton, 4.359 (d, 1H, $J_{\text{gem}} = 11.0$ Hz) partially overlapped with H-3, 4.323 (dd, 1H, $J_{3,2} = 9.4$ Hz, $J_{3,4} = 9.0$ Hz, H-3), 4.148-3.977 (m, 4H, OCH₂CH₂O, OCH₂COOCH₃ (δ 4.100-4.087, 2s, each 1H)), 3.731-3.680 (m, 6H, OCH₂CH₂O, H-5, COOCH₃ (δ 3.693, s, 3H)), 3.470-3.289 (m, 7H, H-2 (δ 3.448, dd, $J_{1,2} = 3.7$ Hz, $J_{2,3} = 9.4$ Hz), H-2', H-3', H-5', OCH₃ (δ 3.289, s, 3H)), 3.153 (dd, 1H, $J_{4',3'} = 8.5$ Hz, $J_{4',5'} = 9.0$ Hz, H-4'), 3.067 (dd, 1H, $J_{4,3} = 9.0$ Hz, $J_{4,5} = 9.5$ Hz, H-4), 1.247 (d, 6H, $J_{6,5} = J_{6',5'} =$

6.0 Hz, H-6, H-6'). Elemental analysis was not attempted due to insufficient material.

Methyl 6-deoxy-3-O-[6-deoxy-3-O-(4-methoxycarbonyl-3-oxabutyl)-β-D-glucopyranosyl]-β-D-glucopyranoside (30). - Compound **57** (30.5 mg, 3.8×10^{-2} mmol) was dissolved in 98% ethanol (2 mL) containing 5% palladium on carbon (29 mg) and was stirred under hydrogen gas for 2 h. The catalyst was removed by filtration and washed with 98% ethanol. The combined filtrate was evaporated to dryness. The residue was then chromatographed on a silica gel column using solvent N as eluent to provide the desired compound **30** which was devoid of ultraviolet absorption in tlc (16 mg, 95%), R_f : 0.26 (solvent N); $[\alpha]_D -31.7^\circ$ (c 1.06, chloroform). $^1\text{H-nmr}$ (D_2O): δ 4.670 (d, 1H, $J_{1,2} = 7.5$ Hz, H-1), 4.367 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1'), 4.263 (s, 2H, $\text{OCH}_2\text{COOCH}_3$), 3.993 (2H, $\text{OCH}_2\text{CH}_2\text{O}$), 3.786-3.752 (5H, $\text{OCH}_2\text{CH}_2\text{OCOOCH}_3$ (δ 3.763, s)), 3.635 (t, 1H, $J_{3,2} = J_{3,4} = 9.2$ Hz, H-3), 3.537 (s, 3H, OCH_3), 3.529-3.395 (m, 4H, H-5, H-5', H-2, H-2' (δ 3.435, dd, 1H, $J_{2',3'} = 9.0$ Hz, $J_{2',1'} = 8.0$ Hz)), 3.358 (t, 1H, $J_{3',2'} = 9.0$ Hz, $J_{3',4'} = 9.0$ Hz, H-3'), 3.260 (dd, 1H, $J_{4',3'} = 9.0$ Hz, H-4'), 3.227 (t, 1H, $J_{4,5} = J_{4,3} = 9.5$ Hz, H-4), 1.301, 1.279 (2d, each 3H, $J_{6,5} = J_{6',5'} = 6.0$ Hz, H-6, H-6'). $^{13}\text{C-nmr}$ (D_2O): δ 173.74 (COOCH_3), 103.68, 103.45 (C-1, C-1'), 85.55, 85.10, 75.15, 74.24, 74.09, 73.62, 72.67, 72.39 (all methine C-2, 2', 3, 3', 4, 4', 5, 5'), 72.08 ($\text{CH}_2\text{COOCH}_3$), 71.55 ($\text{OCH}_2\text{CH}_2\text{O}$), 68.48 ($\text{CH}_2\text{OCH}_2\text{COOCH}_3$), 58.01 (OCH_3), 53.20 (COOCH_3), 17.49, 17.41 (C-6, C-6').

Anal. calc. for $\text{C}_{18}\text{H}_{32}\text{O}_{12} + 0.5 \text{H}_2\text{O}$: C, 48.10; H, 7.18. Found: C, 48.10; H, 7.18.

Methyl 6-deoxy-3-O-[6-deoxy-3-O-(4-methoxycarbonyl-3-oxabutyl)- β -D-glucopyranosyl]- α -D-glucopyranoside (31). - Hydrogenation of compound **58** (4.5 mg, 5.60×10^{-2} mmol) under the conditions described for the conversion of **57** to **30** gave, after chromatographic purification using solvent N as eluent, the title compound **31** as a syrup in quantitative yield, R_f : 0.22 (solvent N). ^1H -nmr (D_2O): δ 4.741 (d, 1H, $J_{1,2} = 3.0$ Hz, H-1), 4.632 (d, 1H, $J_{1',2'} = 7.5$ Hz, H-1'), 4.254 (s, 2H, $\text{OCH}_2\text{COOCH}_3$), 4.005-3.974 (m, 2H, $\text{OCH}_2\text{CH}_2\text{O}$), 1.271 (2d, each 3H, $J_{6,5} = J_{6',5'} = 6.2$ Hz, H-6, H-6'). ^{13}C -nmr (CDCl_3): δ 170.74 (COOCH_3), 104.79, 99.00 (C-1, C-1'), 87.31, 86.26, 74.49, 74.27, 73.99, 72.45, 71.45, 67.57 (all methine C-2, 2', 3, 3', 4, 4', 5, 5'), 71.97 ($\text{CH}_2\text{COOCH}_3$), 71.88 ($\text{OCH}_2\text{CH}_2\text{O}$), 68.24 ($\text{CH}_2\text{OCH}_2\text{COOCH}_3$), 55.20 (OCH_3), 52.10 (COOCH_3), 17.68 (C-6, 6').

1,2,4-Tri-O-acetyl-6-deoxy-3-O-[2,4-di-O-acetyl-6-deoxy-3-O-(4-methoxycarbonyl-3-oxabutyl)- β -D-glucopyranosyl]- α,β -D-glucopyranose (61). - Compound **59** (79.4 mg, 8.80×10^{-2} mmol) was dissolved in 98% ethanol (4 mL) containing 5% palladium on carbon (80 mg) and stirred under hydrogen (1 atm) for 2.5 h, by which time tlc indicated the complete debenzylation of compound **38** to produce a more polar product (R_f : 0.19, solvent A), presumably the mixture of α and β -anomers of compound **60**. The catalyst was removed by filtration, washed with 95% ethanol and the filtrate taken to dryness. The residue was then further dried in an oil pump vacuum and acetylated with acetic anhydride-pyridine (1:2, 1.5 mL) for 18 h at room temperature. Excess acetic anhydride and pyridine were removed by coevaporating with dry toluene (10 mL x 5). The residue was purified by chromatography on silica gel column using solvent R as eluent.

Pure **61** was obtained as a mixture of α and β anomers in a 1:1 ratio (51 mg, 93%) as a white solid (R_f : 0.57, solvent H). ^1H -nmr (CDCl_3): δ 6.182 (d, $J_{1\alpha,2\alpha} = 3.5$ Hz, H-1 α), 5.587 (d, $J_{1\beta,2\beta} = 8.0$ Hz, H-1 β), 5.075 (dd, $J_{2\beta,1\beta} = 8.0$ Hz, $J_{2\beta,3\beta} = 9.0$ Hz, H-2 β), 5.005 (dd, $J_{2\alpha,3\alpha} = 10.0$ Hz, $J_{2\alpha,1\alpha} = 3.5$ Hz, H-2 α), 4.842 (m, H-2' α , H-2' β), 4.776, 4.769 (2t, each 1H, $J_{4'\alpha,5'\alpha} = J_{4'\alpha,3'\alpha} = 9.5$ Hz, $J_{4'\beta,5'\beta} = J_{4'\beta,3'\beta} = 9.5$ Hz, H-4' α , H-4' β), 4.738 (t, $J_{4\beta,5\beta} = J_{4\beta,3\beta} = 9.5$ Hz, H-4 β) overlapping with H-4' α , H-4 α , 4.641 (t, $J_{4\alpha,5\alpha} = J_{4\alpha,3\alpha} = 9.5$ Hz, H-4 α), 4.493 (d, $J_{1'\beta,2'\beta} = 8.0$ Hz, H-1' β), 4.439 (d, $J_{1'\alpha,2'\alpha} = 8.0$ Hz, H-1' α), 4.085 (s, 2H, $\text{OCH}_2\text{COOCH}_3$), 3.985 (t, $J_{3\alpha,4\alpha} = J_{3\alpha,2\alpha} = 9.5$ Hz, H-3 α) partially overlapped with H-5' α , 3.861 (t, $J_{3\beta,4\beta} = J_{3\beta,2\beta} = 9.5$ Hz, H-3 β), 3.734 (s, 3H, COOCH_3), 3.672 (m, > 2H, $\text{OCH}_2\text{CH}_2\text{O}$), 3.574 (t, 2H, $\text{OCH}_2\text{CH}_2\text{O}$), 3.491, 3.472 (2t, each 1 H, $J_{3'\alpha,2'\alpha} = J_{3'\alpha,4'\alpha} = 9.5$ Hz, H-3' α , $J_{3'\beta,2'\beta} = J_{3'\beta,4'\beta} = 9.5$ Hz, H-3' β) partially overlapped with H-5', H-5, 2.166-2.012 (m, 5COCH_3), 1.237-1.175 (m, H-6 α,β and H-6' α,β). ^{13}C -nmr (CDCl_3): δ 170.90 (COOCH_3), 169.70, 169.44, 169.33, 169.24, 169.06, 168.88 (all carbonyl of 5 OCOCH_3), 101.02, 100.86, 91.90, 89.40, 80.85, 80.76, 78.53, 75.79, 74.21, 74.18, 73.00, 72.37, 72.23, 71.81, 71.19, 70.18, 70.08, 68.07 (all methine C-1, 1', 2, 2', 3, 3', 4, 4', 5, 5'), 70.85 ($\text{CH}_2\text{COOCH}_3$), 70.79 ($\text{OCH}_2\text{CH}_2\text{O}$), 68.70 ($\text{CH}_2\text{OCH}_2\text{COOCH}_3$), 51.75 (COOCH_3), 21.01, 20.97, 20.92, 20.85, 20.73, 20.63 (5OCOCH_3), 17.62, 17.57, 17.43 (C-6, C-6').

Anal. calc. for $\text{C}_{27}\text{H}_{40}\text{O}_{17}$: C, 50.94; H, 6.33. Found: C, 51.15; H, 6.17.

2,4-Di-O-acetyl-6-deoxy-3-O- [2,4-di-O-acetyl-6-deoxy-3-O-(4-methoxycarbonyl-3-oxabutyl)- β -D-glucopyranosyl]- α -D-glucopyranosyl bromide (**62**). - A

solution of 45% hydrogen bromide in acetic acid (1 mL) was added to a stirring solution of **61** (51 mg, 8.20×10^{-2} mmol) in dichloromethane (1 mL) at 0°C. After stirring for 15 minutes, the reaction mixture was diluted with cold dichloromethane (50 mL) and poured into ice water. The organic layer was washed with cold saturated NaHCO₃ and water (30 mL x 3, pH = 7), dried (Na₂SO₄) and concentrated to a white solid which showed a single spot on tlc (*R*_f: 0.90, solvent H). Compound **62** was not further characterized but was used directly in the preparation of compound **63**. ¹H-nmr (CDCl₃): δ 6.510 (d, 1H, *J*_{1,2} = 4.2 Hz, H-1), 4.521 (d, 1H, *J*_{1',2'} = 8.0 Hz, H-1'), 4.137-4.044 (m, 4H, OCH₂CH₂O, OCH₂COOCH₃ (δ 4.088, s, 2H)), 3.739 (s, 3H, COOCH₃), 2.188, 2.098, 2.071, 2.007 (4s, each 3H, OCOCH₃), 1.244 (d, 6H, *J*_{6,5} = *J*_{6',5'} = 6.2 Hz, H-6, H-6').

1,5-Anhydro-2,6-dideoxy-3-O-[2,4-di-O-acetyl-6-deoxy-3-O-(4-methoxycarbonyl-3-oxabutyl)-β-D-glucopyranosyl]-D-arabino-hex-1-enitol (63). - To a stirred solution of sodium acetate trihydrate (80 mg, 5.88×10^{-1} mmol) in 50% aqueous acetic acid (190 μL) at -15°C, was added zinc dust (49 mg, 7.50×10^{-1} mmol) followed by a solution of copper sulfate pentahydrate (30 μL, 0.9 g in 2.75 mL water). Stirring was continued at -15°C for a few minutes until the blue colour disappeared. A solution of compound **62** (8.20×10^{-2} mmol) in dry acetic acid (150 μL) was added dropwise at -15°C and stirring was continued for 3 h at -10°C to -5°C. The isolation of glucal **63** was accomplished at low temperature and as fast as possible by adding ice into the reaction mixture while solids were removed by filtration. The combined filtrate was extracted with chloroform (15 mL x 3). The extracts were washed sequentially with cold water (0°C) and cold saturated NaHCO₃. The organic solution was dried (Na₂SO₄) and concentrated to a syrupy

residue. Pure compound **63** was obtained (16 mg, 39%) by chromatographic purification using solvent Q for elution (R_f : 0.37); $[\alpha]_D -62.2^\circ$ (c 0.50, chloroform). The molecular weight of compound **63** was confirmed by mass FAB spectra (MW = 518). ^1H -nmr (CDCl_3): δ 6.415 (dd, 1H, $J_{1,3} = 1.3$ Hz, $J_{1,2} = 6.3$ Hz, H-1), 5.068 (ddd, 1H, $J_{4,5} = J_{4,3} = 4.75$ Hz, $J_{4,2} = 0.7$ Hz, H-4), 4.922 (dd, 1H, $J_{2',3'} = 9.7$ Hz, $J_{2',1'} = 8.0$ Hz, H-2'), 4.804 (t, 1H, $J_{4',5'} = J_{4',3'} = 9.4$ Hz, H-4') partially overlapped with H-2, 4.784 (ddd, 1H, $J_{2,1} = 6.3$ Hz, $J_{2,3} = 3.4$ Hz, $J_{2,4} = 0.7$ Hz, H-2), 4.543 (d, 1H, $J_{1',2'} = 8.3$ Hz, H-1'), 4.187 (qdd, 1H, $J_{5,6} = 6.7$ Hz, $J_{5,4} = 4.75$ Hz, $J_{5,3} = 1.3$ Hz, H-5), 4.137 (dddd, 1H, $J_{3,4} = 4.0$ Hz, $J_{3,5} = 1.3$ Hz, $J_{3,2} = 3.75$ Hz, $J_{3,1} = 1.3$ Hz, H-3), 4.106 (s, 2H, $\text{OCH}_2\text{COOCH}_3$), 3.742 (s, 3H, COOCH_3), 3.711, 3.593 (2ddd, each 2H, $\text{OCH}_2\text{CH}_2\text{O}$), 3.532 (t, 1H, $J_{3,4} = J_{3,2} = 9.2$ Hz, H-3), 3.463 (qd, 1H, $J_{5',6'} = 6.2$ Hz, $J_{5',4'} = 9.5$ Hz, H-5'), 2.098, 2.076, 2.063 (3s, each 3H, OCOCH_3), 1.294 (d, 3H, $J_{6,5} = 6.7$ Hz, H-6), 1.212 (d, 3H, $J_{6',5'} = 6.2$ Hz, H-6'). ^{13}C -nmr (CDCl_3): δ 169.81, 169.72, 169.13 (3OCOCH_3) 145.21 (C-1), 98.99, 97.54, 80.983, 74.27, 72.67, 72.21, 72.09, 70.98, 70.24 (all methine C-1', 2, 2', 3, 3', 4, 4', 5, 5'), 71.10 ($\text{CH}_2\text{COOCH}_3$), 70.86 ($\text{OCH}_2\text{CH}_2\text{O}$), 68.71 ($\text{CH}_2\text{OCH}_2\text{COOCH}_3$), 51.76 (COOCH_3), 21.09, 20.94, 20.89 (3OCOCH_3), 17.48, 16.18 (C-6, C-6').

15-Anhydro-3-O-[3-O-(4-carboxy-3-oxabutyl)-6-deoxy- β -D-glucopyranosyl]-2,6-dideoxy-D-arabino-hex-1-enitol (32). - Compound **63** (12 mg, 2.32×10^{-2} mmol) was dissolved in dry methanol (300 μL). To this solution was added a sodium methoxide solution (150 μL , 0.5 N) and the mixture was stirred for 3 h at room temperature. Tlc showed the complete conversion of compound **63** (R_f : 0.52,

solvent H) to two more polar products: the major product with R_f : 0.11 (solvent H), presumably compound **64**, and the minor product with R_f : 0 (solvent H) or R_f : 0.55 (solvent M) which was identified as compound **32**. Stirring was continued for an additional 15 h; tlc revealed the complete conversion of compound **64** to compound **32**. The reaction mixture was neutralized with Amberlite IRC-50(H^+) resin at $-10^\circ C$. The resin was removed by filtration and the solvent was evaporated to provide **32** as a clear syrup in quantitative yield. A portion of compound **32** (4.0 mg) after passage through a column of Bio-gel (10% ethanol) and lyophilization of the eluate gave a white powder (3.0 mg; 75%), $[\alpha]_D -70.0^\circ$ (c 0.10, H_2O). ^1H -nmr (D_2O): δ 6.451 (dd, 1H, $J_{1,2} = 6.3$ Hz, $J_{1,3} = 1.3$ Hz, H-1), 4.941 (dd, 1H, $J_{2,1} = 6.2$ Hz, $J_{2,3} = 2.6$ Hz, H-2), 4.611 (m, 1H, $J_{1'2'} = 8.0$ Hz, H-1', virtually coupled to H-3'), 4.348 (ddd, 1H, $J_{3,4} = 6.7$ Hz, $J_{3,2} = 2.6$ Hz, $J_{3,1} = 1.3$ Hz, H-3), 4.015-3.943 (m, 5H, H-5, OCH_2CH_2O (δ 4.002, 2H, $OCHCH_2O$, 3.960, s, 2H, OCH_2COOH)) 3.716 (m, 2H, OCH_2CH_2O), 3.625 (dd, 1H, $J_{4,5} = 9.2$ Hz, $J_{4,3} = 6.7$ Hz, H-4), 3.513 (qd, 1H, $J_{5',4'} = 9.2$ Hz, $J_{5',6'} = 6.2$ Hz, H-5'), 3.378-3.332 (m, 2H, H-2', H-3'), 3.304-3.242 (m, 1H, $J_{4',5'} = 9.2$ Hz, H-4'), 1.356 (d, 3H, $J_{6,5} = 6.2$ Hz, H-6), 1.302 (d, 3H, $J_{6',5'} = 6.2$ Hz, H-6'). ^{13}C -nmr (D_2O): δ 145.61 (C-1), 101.02, 100.27 (C-2, C-1'), 85.33, 77.33, 75.48, 75.17, 73.58, 72.61, 72.53 (all methine C-2', 3, 3', 4, 4', 5, 5'), 72.18 (CH_2COOH), 70.88 (OCH_2CH_2O), 70.27 (CH_2OCH_2COOH), 17.44, 17.06 (C-6, 6').

REFERENCES

1. A. Fersht, *Enzyme Structure and Mechanism* (Freeman, San Francisco, ed. 2, 1985), p. 331.
2. L. Pauling, *Chem. Eng. News*, **24**, 1375 (1946); *Nature*, **161**, 707 (1948).
3. W.P. Jencks, *Adv. Enzymol. Relat. Areas Mol. Biol.*, **43**, 219 (1975).
4. L. Pauling, "Chemical Achievement and Hope for the Future." *Am. Sci.*, **36**, 51 (1948).
5. W.P. Jencks, *Catalysis in Chemistry and Enzymology* (McGraw Hill, New York) p. 354 and 524 (1969).
6. A. Joseph, *Chemical Week*, August 12, **36** (1987).
7. R.A. Lerner, A. Tramontano, *Trends Biochem. Sci.*, **12**, 427 (1987).
8. J. Jacobs, P.G. Schultz, R. Sugasawara, M. Powell, *J. Am. Chem. Soc.*, **109**, 2174 (1987).
9. A. Tramontano, K.D. Janda, R.A. Lerner, *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 6736 (1986).
10. A. Tramontano, K.D. Jana, R.A. Lerner, *Science*, **234**, 1566 (1986).
11. S.J. Pollack, J.W. Jacobs, P.G. Schultz, *Science*, **234**, 1570 (1986).
12. A.D. Napper, S.J. Benkovic, A. Tramontano, R.A. Lerner, *Science*, **237**, 1041 (1987).
13. P.G. Schultz, *Acc. Chem. Res.*, **22**, 287 (1989).
14. P.G. Schultz, *Science*, **240**, 426 (1988).
15. K.D. Janda, R.A. Lerner, A. Tramontano, *J. Am. Chem. Soc.*, **110**, 4835 (1988).
16. S.J. Benkovic, A.D. Napper, R.A. Lerner, *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 5355 (1988).

17. A.W. Schwaba, M.I. Weinhouse, R.A. Lerner, *J. Am. Chem. Soc.*, **111**, 2344 (1989).
18. A. Tramontano, A.A. Ammann, R.A. Lerner, *J. Am. Chem. Soc.*, **110**, 2282 (1988).
19. K.D. Janda, D. Schloeder, S.J. Benkovic, R.A. Lerner, *Science*, **241**, 1188 (1989).
20. D. Hilvert, S.H. Carpenter, K.D. Nared, M.T.M. Anditor, *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 4953 (1988).
21. D. Hilvert and K.D. Nared, *J. Am. Chem. Soc.*, **110**, 5593 (1988).
22. D.Y. Jackson, P.G. Schultz, J.W. Jacobs, R. Sugasawara, S.H. Reich, P.A. Bartlett, *J. Am. Chem. Soc.*, **110**, 484 (1988).
23. S.D. Copley, J.D. Knowles, *J. Am. Chem. Soc.*, **107**, 5306 (1985).
24. W.J. Guifford, S.D. Copley, J.D. Knowles, *J. Am. Chem. Soc.*, **109**, 5013 (1987).
25. R.A. Lerner, *Adv. Immunol.*, **36**, 1 (1984).
26. C.A. Vernon, *Proc. Roy. Soc. (London) B*, **167**, 389 (1967).
27. G.A. Levvy, S.M. Snaith, *Adv. Enzymol.*, **36**, 151 (1972).
28. H.M. Flowers and N. Sharon, *Adv. Enzymol.*, **48**, 29 (1979).
29. E.T. Reese, F.W. Parrish, M. Ettlinger, *Carbohydr. Res.*, **18**, 381 (1971).
30. H.M. Dettinger, G. Kurg, J. Lehman, *Carbohydr. Res.*, **74**, 301 (1979).
31. D.F. Wentworth, R. Wolfenden, *Biochem.*, **13**, 4715 (1974).
32. Y.C. Lee, *Biochem. Biophys. Res. Comm.*, **35**, 161 (1969).
33. C.C.F. Blake, L.N. Johnson, G.A. Mair, A.C.T. North, D.C. Phillip, V.R. Sarma, *Proc. Roy. Soc. (London) B*, **167**, 378 (1967).
34. D.C. Phillip, *Scientific American*, **215**, 78 (1966).

35. I.I. Secemski, G.E. Lienhard, *J. Am. Chem. Soc.*, **93**, 3549 (1971).
36. L.N. Johnson, D.C. Phillips, *Nature*, **206**, 761 (1965).
37. J. Conchie, A.J. Hay, I. Strachan, G.A. Levvy, *Biochem.*, **102**, 929 (1967).
38. Z. Walaszek, D. Horton, I. Ekiel, *Carbohydr. Res.*, **106**, 193 (1982).
39. R.U. Lemieux, *Chem. Soc. Rev.*, **18**, 347 (1989).
40. H. Thøgersen, R.U. Lemieux, K. Bock, B. Meyer, *Can. J. Chem.*, **60**, 44 (1982).
41. K. Bock, *Pure & Appl. Chem.*, **55**, 605 (1983).
42. R.E. Tenbrink, *J. Org. Chem.*, **52**, 418 (1987).
43. O. Th. Schmidt, in *Methods in Carbohydrate Chemistry*, Vol. II, 322, Procedure A, (Eds.) R.L. Whistler and M.L. Wolfrom, Academic Press, 1963.
44. A.S. Meyer, T. Reichstein, *Helv. Chim. Acta*, **29**, 152 (1946).
45. S.H. Tahir, O. Hindsgaul, *Can. J. Chem.*, **64**, 1771 (1986).
46. O.P. Srivastava, O. Hindsgaul, *Carbohydr. Res.*, **179**, 137 (1988).
47. B. Helferich and K.-F. Wedemeyer, *Justus Liebigs Ann. Chem.* **563**, 139-145 (1949).
48. E.J. Corey, J.W. Suggs, *J. Org. Chem.*, **38**, 3224 (1973).
49. R. Gigg, C.D. Warren, *J. Chem. Soc.*, (C) 1903 (1968).
50. S. Fliszár, M. Granger, *J. Am. Chem. Soc.*, **92**, 3361 (1970).
51. Y. Oikawa, T. Yoshioka, O. Yomemitsu, *Tetrahedron Letters*, **23**, 885 (1982).
52. B. Classon, P.J. Garegg, B. Samuelsson, *Acta Chemica Scandinavia B*, **38**, 419 (1984).
53. R. Johansson, B. Samuelsson, *J. Chem. Soc. Perkin Trans. 1*, 2371 (1984).

54. R. Roth and W. Pigman, in *Methods in Carbohydrate Chemistry*, Vol. II, 405 (Eds.) R.L. Whistler and M.L. Wolfrom, Academic Press, 1963.
55. W. Korytnyk, N. Angelino, O. Dodson-Simmons, H. Hanchak, M. Madson, S.V. Horvath, *Carbohydr. Res.*, **113**, 166 (1983).
56. L.D. Hall, L.F. Johnson, *Tetrahedron*, **20**, 883 (1964).
57. A.A. Chalmers, R.H. Hall, *J. Chem. Soc. Perkin Trans.* **2**, 728 (1974).
58. K. Vangehr, P. Luger, H. Paulsen, *Carbohydr. Res.*, **70**, 1 (1979).
59. R.U. Lemieux, D.R. Bundle, D.A. Baker, *Can. J. Biochem.*, **56**, 1102 (1978).
60. D.D. Perrin, W.L. Armarego, D.R. Perrin, "*Purification of Laboratory Chemicals*", Pergamon Press, London, 2nd ed., (1980).
61. G.W. Kramer, A.B. Levy, M. Midland, "*Organic Synthesis via Boranes*", McGraw-Hill, New York, Chapter 9, (1972).
62. D.E. Hoiness, C.P. Wade, S.P. Rowland, *Can. J. Chem.*, **46**, 667 (1968).